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AN EFFECT OF PROGESTERONE ON ESTROGEN-INDUCED HYPERCALCEMIA IN THE SEXUALLY IMMATURE PULLET[1]

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[Received for publication April 3, 1958]

ABSTRACT

Sexually immature crossbred pullets were given daily intramuscular injections of 0.5 mgm. estradiol benzoate (ODB) for 14 days. Food intake was kept uniform as between pullets. Concurrent administration of 0.025 mgm., 0.05 mgm. or 0.10 mgm. progesterone (PGST) did not affect the hypercalcemia induced by the ODB, although these levels of PGS Taugmented the increase of oviduct weight induced by the estrogen. Administration of 0.5 mgm. PGST was found to augment significantly the hypercalcemia, but did not modify the increase of oviduct weight induced by estrogen.

In a second similar experiment the hypercalcemia induced by 0.5 mgm. ODB daily for 14 days was not affected by 0.05 mgm. or 0.10 mgm. PGST daily, although these levels of PGST augmented significantly the estrogen-induced increase of oviduct weight. Administration of 0.50 mgm. PGST daily increased the estrogen-induced hypercalcemia significantly but an increase with 1.0 mgm. PGST attained significance only at $P = 0.10$. As the dosage of PGST increased, oviduct weight increased to a maximum and then declined.

INTRODUCTION

Riddle and Lahr (17) reported that progesterone (PGST) had enhanced estrogen-induced hypertrophy of the ring-dove's oviduct. Hertz *et al.* (7, 8) reported a depressant effect of PGST on estrogen-induced hypertrophy of the chick's oviduct. Similar depressant effects of PGST on response of the oviduct to estrogen have been reported by others (1, 13). Mason (11) found that PGST may enhance the response of the chick's oviduct to estrogen and further demonstrated that the effect of PGST on the response of the chick's oviduct was dependent on (a) the level of dosage with estrogen, and (b) the ratio of the dosage of estrogen to dosage of progesterone. Breneman (5) has studied the interaction of steroid hormones on pullets that were 40 days of age when killed at the end of his experiments. Large dosages of PGST (0.5 mgm. daily) greatly enhanced the effect of low dosages of estradiol dipropionate (0.025 mgm. daily) on the oviduct.

It seems clear that the effect of PGST on the oviduct will vary with levels and ratios of estrogen and PGST used and probably also with the age

[1]Contribution from the Faculty of Agriculture, McGill University, Macdonald College, Que. Journal Series No. 388.

of the bird. At low doses of estrogen, PGST will enhance oviduct growth. At higher doses of estrogen, small doses of PGST will enhance the hypertrophy of the oviduct, but larger doses of PGST will depress it.

In previous experiments, where PGST depressed estrogen-induced hypertrophy of the oviduct, there was no evidence of any effect of the PGST on the estrogen-induced hypercalcemia or the size or composition of the liver of the estrogenized pullet (7, 15). It seemed desirable to extend these observations so as to include levels of ODB and PGST such that the enhancing effect of PGST on response to ODB might be in evidence. The present paper describes two experiments directed to that end.

MATERIALS AND METHODS

Experiment 1

Forty-two crossbred (New Hampshire x Barred Plymouth Rock) sexually immature pullets of the same strain and hatching were placed at random in individual cages and assigned at random between six groups as set out in Table 1. Group 1 served as control and was given daily intramuscular injections of a volume of sesame oil solvent equal to that injected into the other groups. Group 2 received 14 daily doses of 0.5 mgm. estradiol benzoate (ODB) (as 'Progynon', Schering) in sesame oil. The other four groups received the same dosage of estrogen but in admixture with PGST (as 'Proluton', Schering) at levels ranging from 0.025 mgm. daily to 0.500 mg. daily. All injections were made into the breast muscle. It was considered not necessary to include groups receiving PGST only, because the observations of Bolton (1, 2) had shown that the effects of 1 mgm. PGST by itself every other day for 12 days on the weight of the oviduct were very slight, if any.

Daily food intake was strictly controlled so that all the birds ate the same amount of food on any day.

On the morning of the 15th day the birds were decapitated and bled. Livers and kidneys were removed and weighed. Serum Ca was determined by the method of Halverson as described by Peters and Van Slyke (14). Samples (5-10 gm.) of the livers were disintegrated in 10 per cent trichloroacetic acid in a Waring Blendor. The mixture was filtered, and the residue was defatted and dried with alcohol and ether. The protein (N x 6.25) was determined on the final fat-free residue by macro-kjeldahl determinations.

Experiment 2

The experimental birds comprised 40 sexually immature pullets similar to those in Experiment 1. The same experimental technique and analytical methods were used as in Experiment 1. The birds were assigned between five groups as set out in Table 2. All the pullets received a daily dose of 0.5 mgm. ODB. Group A received ODB only, and the remaining four groups received PGST at levels ranging from 0.05 mgm. to 1.0 mgm. daily (see Table 2). A negative control group was not included because the effects of ODB alone were considered to be sufficiently established already.

TABLE 1.—EFFECTS OF PROGESTERONE ON THE RESPONSES OF THE SEXUALLY IMMATURE PULLET TO ESTROGEN. AVERAGE RESULTS. DURATION OF HORMONAL TREATMENT: 14 DAYS. SEVEN BIRDS PER GROUP

Group	1	2	3	4	5	6
ODB, daily dose, mgm.	nil	0.5	0.5	0.5	0.5	0.5
PGST, daily dose, mgm.	nil	nil	0.025	0.050	0.100	0.500
Live wt., initial, gm.	740 ± 9	736 ± 21	747 ± 15	742 ± 11	707 ± 16	752 ± 23
Live wt., final, gm.	1054 ± 14	1014 ± 19	1019 ± 24	1009 ± 21	1013 ± 19	976 ± 58
Live wt., gain, gm.	314 ± 14	278 ± 16	272 ± 14	267 ± 17	306 ± 15	224 ± 35
Total feed intake per bird, kgm.*	0.98	0.98	0.98	0.98	0.98	0.98
Ovary, mgm.	206 ± 16	196 ± 6	187 ± 7	187 ± 10	187 ± 8	198 ± 16
Oviduct, gm.	0.16 ± 0.02	8.4 ± 0.61	11.3 ± 1.03	12.2 ± 0.81	12.4 ± 0.61	9.7 ± 0.64
Serum Ca, mgm./100 ml.	12.4 ± 0.22	29.5 ± 2.63	29.9 ± 4.15	30.9 ± 2.05	35.6 ± 4.94	42.0 ± 2.33
Liver, gm.	3.3 ± 0.12	4.1 ± 0.17	3.9 ± 0.14	4.0 ± 0.13	4.2 ± 0.13	4.5 ± 0.18
Kidneys, gm.	6.5 ± 0.30	7.1 ± 0.19	7.1 ± 0.16	6.4 ± 0.12	7.3 ± 0.57	6.8 ± 0.58

NOTES: *Daily food intake controlled so that all birds consumed the same weight of food.
The figures following the ± signs denote the standard deviation of the means.

TABLE 2.—EFFECTS OF PROGESTERONE ON RESPONSES OF THE SEXUALLY IMMATURE PULLET TO ESTROGEN. AVERAGE RESULTS. DURATION OF HORMONAL TREATMENT: 14 DAYS. EIGHT BIRDS IN EACH GROUP

Group	A	B	C	D	E
ODB, daily dose, mgm.	0.5	0.5	0.50	0.500	0.500
PGST, daily dose, mgm.	nil	0.050	0.250	0.500	1.000
Live wt., initial, gm.	928 ± 21	938 ± 18	947 ± 18	910 ± 17	925 ± 10
Live wt., final, gm.	1151 ± 27	1159 ± 16	1182 ± 29	1126 ± 12	1123 ± 15
Live wt., gain, gm.	222 ± 11	222 ± 7	234 ± 12	203 ± 5	198 ± 16
Total food intake per bird, kgm.*	1.01	1.01	1.01	1.01	1.01
Ovary, mgm.	249 ± 16	225 ± 16	206 ± 20	247 ± 16	216 ± 10
Oviduct, gm.	9.6 ± 0.47	13.0 ± 0.79	16.3 ± 0.72	11.4 ± 0.39	8.2 ± 0.36
Serum Ca, mgm./100 ml.	28.8 ± 3.1	32.3 ± 3.0	28.0 ± 3.9	40.4 ± 3.5	36.0 ± 2.6
Liver, gm.	31.2 ± 1.1	29.4 ± 1.0	30.0 ± 1.1	30.9 ± 1.0	28.2 ± 0.6
Liver protein, gm.	5.7 ± 0.19	5.6 ± 0.14	5.7 ± 0.13	5.6 ± 0.19	5.6 ± 0.13
Kidneys, gm.	9.4 ± 0.17	9.1 ± 0.20	9.2 ± 0.26	9.2 ± 0.28	9.2 ± 0.22

NOTES: *Daily food intake controlled so that all birds consumed the same weight of food.
The figures following the ± signs denote the standard deviation of the means.

RESULTS

Experiment 1

The average results of Experiment 1 are presented in Table 1. There were not any significant group differences in the initial or the final live weights. The live weight gain of Group 1 was significantly greater than that of either Group 3 or 4 or 6. There were not any significant differences as between the live weight increases of Groups 2, 3, 4, 5 and 6. Thus the estrogen treatment slightly decreased the rate of live weight gain over the short term of this experiment, while PGST treatments did not significantly affect this response. In this connection, it is to be remembered that food intake was kept the same for all groups.

The ODB treatment of itself gave an average oviduct weight of 8.4 gm. This weight is similar to that observed in this laboratory in other experiments where similar pullets were treated in the same way. Administration of 0.025 mgm., 0.050 mgm. or 0.100 mgm. PGST increased significantly the response of the oviduct to estrogen, but 0.500 mgm. PGST did not increase the degree of estrogen-induced hypertrophy of the oviduct. The statistical significance of these effects may be shown by the following "t" values for certain group comparisons:—

Comparison	t(12 d.f.)
Group 2 vs. Group 3	2.39*
Group 2 vs. Group 4	3.72**
Group 2 vs. Group 5	4.55**
Group 2 vs. Group 6	0.95

* Significant at P = 0.05
** Significant at P = 0.01

These results for the response of the oviduct weight are in general conformity with Mason's observations (11), and it is probable that a higher level of PGST would have reduced the weight of the oviducts of the estrogenized birds (see results of Experiment 2 below).

The main interest of the experiment lay in the results for serum Ca. Comparison of Groups 1 and 2 showed that 0.5 mgm. ODB per day had given a degree of hypercalcemia of the same order as that observed as a result of the same treatment in other experiments in this laboratory. Comparison of Group 2 with the groups treated with PGST showed that the three lower levels of PGST did not increase significantly the serum Ca level, but that the highest level of PGST (0.500 mgm.) gave a highly significant augmentation of the hypercalcemia. The relevant significances were as follows:—

Comparison	t(12 d.f.)
Group 1 vs. Group 2	6.47**
Group 2 vs. Group 3	0.07
Group 2 vs. Group 4	0.41
Group 2 vs. Group 5	1.09
Group 2 vs. Group 6	3.56**

** Significant at P = 0.01

These results leave little doubt that the highest level of PGST (0.500 mgm.) in this experiment enhanced the response of serum Ca to 0.5 mgm. ODB daily.

The ODB treatment produced the usual significant increases in liver weight and liver protein, but there were no significant differences in liver weight or liver protein between Groups 2, 3, 4, 5 and 6.

Experiment 2

The average results of Experiment 2 are set out in Table 2.

None of the differences in initial or final live weights attained significance. However, the average live weight gain of Group C was significantly greater than that of Group D (P = 0.05) and significantly greater than that of Group E at P = 0.10. These results suggest that the higher doses of PGST depressed the live weight gains of estrogenized pullets under the given conditions of uniform limited food intake and short-term experimental period.

None of the differences in ovary weight attained significance, but the effects on oviduct weight were significant and in close agreement with those of Experiment 1. The significances of certain comparisons of average oviduct weights were as follows:—

Comparison	t(14 d.f.)
Group A vs. Group B	3.66**
Group A vs. Group C	7.82**
Group A vs. Group D	2.88*
Group A vs. Group E	2.30*
Group D vs. Group E	5.94*

* Significant at P = 0.05
** Significant at P = 0.01

The oviducts of Group C were significantly heavier than those of any other group, so that the maximal enhancement of the effect of 0.5 mgm. ODB daily on the oviduct weight was obtained with 0.250 mgm. PGST daily. The oviducts of Group 5 were significantly lighter than those of Group A, so that 0.500 mgm. PGST had definitely depressed the response of the oviduct to 0.5 mgm. ODB daily.

The results for the effect of PGST on estrogen-induced hypercalcemia showed that neither of the two lower levels of PGST affected the degree of hypercalcemia significantly. The 0.500 mgm. level of PGST enhanced the hypercalcemia significantly (P = 0.05). The 1.0 mgm. level of PGST enhanced the hypercalcemia slightly but this effect was of low significance. The significances were as follows:—

Comparison	t(14 d.f.)
Group A vs. Group B	0.80
Group A vs. Group C	0.17
Group A vs. Group D	2.48*
Group A vs. Group E	1.72

* Significant at P = 0.05

These results confirm those of Experiment 1, therefore, as far as the effect of PGST on the estrogen-induced hypercalcemia is concerned. There were no significant differences between Groups A, B, C and D in respect of liver weight, but Group E had significantly lighter livers than either Group A or Group D. None of the differences in liver protein or kidney weight attained significance.

DISCUSSION

The results of these two experiments may be summarized as follows:—

(a) The hypercalcemia of sexually immature pullets induced by 0.5 mgm. ODB per day was not affected by PGST at dosage levels that were able greatly to enhance estrogen-induced hypertrophy of the oviduct;

(b) The hypercalcemia induced by 0.5 mgm. ODB daily was enhanced by PGST at higher dosage levels, viz., 0.5 mgm. daily and upward, that either did not enhance hypertrophy of the oviduct as much as did lower levels of PGST, or that depressed the estrogen-induced hypertrophy of the oviduct.

Exogenous PGST is known to affect the secretory activity of the magnum of oviducts hypertrophied as a result of treatment with estrogen (3, 4). Progesterone itself has been detected in extracts of ovaries of laying hens (9) and more recently it has been shown that small amounts are present in the blood draining from the ovaries of the laying hen (10). It is highly probable that progesterone action is implicated in the physiology of normal avian reproduction, even if the precise roles have not as yet been fully clarified. It is known that testosterone propionate will enhance the response of the pullet's oviduct to estrogen (6). There is some evidence that PGST is converted to androgen in the bovine and in the ram (16), and certain androgens have been identified in the feces of the pregnant cow following administration of PGST (13). It has also been established that PGST may be converted *in vitro* to testosterone and Δ4-androstene-3, 17-dione by rat testicular tissue (18) and to Δ4-androstenedione by human fetal adrenal tissue (19). Although there is not any comparable evidence for conversion of PGST to androgens by tissues of the fowl, the possibility of such conversions exists. On the other hand, maximal augmenting effect on oviduct hypertrophy in the present experiments was obtained with low levels of PGST that would not be expected, a priori, to yield androgen as plentifully as the higher doses, and yet higher doses antagonized the effect of ODB on the oviduct weight. As far as the positive effect of PGST on estrogen-induced hypercalcemia observed in these experiments is concerned, the position is obscure, and it is not yet possible to offer any coherent hypothesis to account for these effects.

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OBSERVATIONS ON THE HISTOPATHOLOGICAL CHANGES CAUSED BY THE LARVAE OF *HYPODERMA BOVIS* (L.) AND *HYPODERMA LINEATUM* (DE VILL.) (DIPTERA: OESTRIDAE) IN TISSUES OF CATTLE

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[Received for publication May 22, 1959]

ABSTRACT

The lesions in submucosal and adventitial connective tissue of the oesophagus of cattle caused by the migrating larvae of *Hypoderma lineatum* (De Vill.), consist of pseudocystic areas and tracks of a gelatinous fibrinous exudate containing necrotic cellular debris and collagen fibres in various stages of dissolution surrounded by accumulations of eosinophils and lymphocytes. The larvae feed on the dissolved connective tissue. These changes suggest that migration through the tissues is mainly by enzymic dissolution of collagen fibres and ground substance. Similar changes were found in the connective tissue surrounding the muscles of "licked beef". The larvae of *H. bovis* (L.) in the epidural fat cause areas of fat necrosis and sometimes invade the periosteum and dura. The greenish colour of the tracks is due to infiltrations of eosinophils. The warble shows a mixed picture of chronic granulomatous and non-suppurative inflammation, encapsulated by dense fibrous tissue. The larva is surrounded by a vascular layer of granulation tissue infiltrated with eosinophils, large mononuclears and plasma cells. Numerous giant cell systems and pseudotubercles are present. The larvae feed on the exudate within the warble cavity. Areas of suppuration are rarely found. The healing of the warble and the reaction to dead larvae are described.

INTRODUCTION

The life histories of the northern cattle grub, *Hypoderma bovis* (L.) and the common cattle grub, *H. lineatum* (De Vill.), have been reviewed by several authors (3, 20, 36). The adult flies deposit their eggs on the hairs of the legs and under-portions of cattle. The first-instar larvae emerge and burrow through the skin to the subcutaneous connective tissue and then begin a remarkable migration through the connective tissues of the host. Many of the larvae of *H. lineatum* migrate to the oesophageal submucosa, whereas the larvae of *H. bovis* migrate to the epidural space of the neural canal. The details of this complex migration have not been worked out. The larvae finally reach the subcutaneous connective tissue of the back of the cattle where they penetrate the hide, moult, and incite an inflammatory reaction which causes localized swellings called warbles. The larvae grow and moult to the third-instar within these swellings which have been called encystment sacs. Strictly, these sacs are not true cysts because they are not lined by epithelium but are chronic abscess cavities containing a foreign body, the warble-fly larva. From these cavities the mature larvae emerge, fall to the ground, burrow a little and pupate.

Curtice (6) and Stub (41, 42) were the first to describe an oesophageal stage of what they thought was *H. bovis*, and Laake (19) specifically described this stage for *H. lineatum*. Hadwen and Fulton (13) found

these larvae in the connective tissue of the diaphragm, duodenum, oesophagus, pericardial sac, and the heart. Similarly, Ono and Yamasaki (32) found oedematous areas in the pericardium, pleura, serous membrane surrounding the oesophagus and trachea, and in the oesophageal submucosa. In addition to the above situations, Malkani (24) found larvae overlying the rumen and spleen, in the connective tissue of the ribs, and sub-peritoneally near the oesophagus. Simmons (39) found larvae typically in the oesophageal submucosa. These observations are valuable in the interpretation of the route taken by the first-instar larvae to the connective tissue of the back, and will be discussed later.

Hinrichsen (14), in 1888, discovered the first-instar larvae of *H. bovis* in the spinal canal of cattle and since that time several authors have reported their presence in this region (4, 10, 18, 34, 35). No account has been given of the pathological changes around larvae in the spinal canal. Kettle and Utsi (16), in post-mortems of two reindeer killed by damage to the central nervous system by migrating larvae of *H. diana* Brauer, found a red, gelatinous material in the epidural space which they regarded as containing blood and cerebrospinal fluid.

Knipling (17) showed that migrating larvae are all in the first stadium and that moulting occurs after the perforation of the hide. It is generally held that the larvae of both *H. bovis* and *H. lineatum*, on reaching the connective tissue of the back, perforate the hide by enzymatic digestion (27, 29, 39, 40). This opinion is based on circumstantial evidence.

The formation of "encystment sacs" around hypodermal larvae following perforation of the hide have been described by Bishopp, Laake, Brundrett, and Wells (3), and others (3, 7, 25), in general terms. O'Flaherty and McLaughlin (27), 25 years ago, published a series of photographs of tissues damaged by the hypodermal stage but made little attempt at interpretation. Simmons (38) reported certain histological changes produced in the oesophagus by the larvae, as well as observations on the formation of the "cysts of dense connective tissue". He related the paucity of infiltration of the tissues with neutrophils to either a negative chemotaxis of the cells to the secretions of the larvae or to the lack of bacterial infection. Ono (29, 30, 31), and Terada and Ono (43), in a series of interesting investigations, reported the secretion by the larval gut of a "hypodermatotoxin" with enzyme-like properties which they thought caused the pathological reaction in the tissues. Ono concluded that the larvae feed on the tissue products of the action of this material. Recently, Lienert and Thorsell (21, 22) have shown that autolysates of migrating larvae contain a powerful collagenase and suggested that the larvae progress through the tissues by the combined action of larval movements and enzymic dissolution of the loose connective tissue. Nenyukov (26) reported on the role of natural enzymes and microflora in the digestion of the "pus" in warble cavities and made a qualitative study of the digestive enzymes secreted by *H. lineatum* and found proteolytic, lipolytic, and glycogenolytic enzymes. Ono (28) stated that the larvae ingest pus but gave no details of its cellular composition.

The new approach to the control of the larvae of *H. bovis* and *H. lineatum* (1, 5, 23) is based on the killing of the migratory and hypodermal

stages of the larvae by insecticides which when administered orally, subcutaneously, or topically penetrate to the larvae in the connective tissue. This work has emphasized the inadequacy of present knowledge on the development and migration of these parasites, the complex host-parasite relationship, and the reactions, both local and systemic, by the host to their presence. This paper reports observations of some of the pathological reactions in the tissues of cattle produced by the first-instar larvae during their migration and by subsequent instars within the warbles.

MATERIALS AND METHODS

The material was obtained from slaughtered cattle at abattoirs near Guelph, Ontario, and from the Entomology Laboratory, Canada Department of Agriculture, Kamloops, British Columbia. Many carcasses of cattle were examined immediately after the animals had been slaughtered. Warbles, pieces of "licked beef", larval tracks in connective tissue, epidural fat, oesophageal submucosa, perioesophageal connective tissue and visceral connective tissue containing first-instar larvae and their tracks, were carefully excised, fixed in Zenker's fluid, embedded in Tissuemat, and sectioned by standard methods. Heidenhain's and Eosin, Masson's Trichrome, Phloxine-Azure II—Methylene Blue, and Eosin—Methylene Blue preparations were made. Smears of the exudate from warble cavities were stained with Gram's stain or Giemsa.

OBSERVATIONS

The Oesophageal Stage of H. lineatum

First-instar larvae of *H. lineatum* were most frequently found lying longitudinally in the loose connective tissue of the submucosa of the posterior third of the oesophagus, from October to January. The anterior end of the larvae sometimes pointed towards the pharyngeal and sometimes towards the gastric end of the oesophagus but was always in the longitudinal axis. The internal layer of circular muscle, together with the external layer of longitudinal muscle (i.e., the tunica muscularis), stripped off easily from the submucosa of the oesophagus revealing the larvae as small swellings in the connective tissue. Greenish tracks of a gelatinous consistency often extended from the swellings towards the junction of the oesophagus and rumen. Sections through these swellings showed the larval cuticle separated by a thin layer of translucent, greenish-yellow exudate from the localized area of oedematous connective tissue. Small haemorrhages were sometimes visible. Occasionally, larvae were found in the more vascular lamina propria in which case they caused small swellings into the oesophageal lumen. Larvae and their tracks were sometimes found in the adventitial connective tissue surrounding the oesophagus, within the pretracheal fascia in the cervical region and also in the endothoracic fascia beneath the parietal pleura.

The microscopic picture of tissues around these larvae and tracks showed several interesting features. The connective tissue immediately surrounding the larvae was oedematous, hyperaemic, and only mildly

infiltrated with eosinophils. There was little fibroblastic reaction. The collagen fibres were swollen, irregularly arranged, and in many areas partially dissolved. A thin layer of exudate containing fibrin strands, a few erythrocytes, eosinophils, and lymphocytes separated the larval cuticle from the swollen connective tissue. Part of this acidophilic exudate extended into the surrounding connective tissue and in these areas the collagen fibres and ground substance were completely dissolved (Figures 1 and 2). Small pieces of incompletely dissolved collagen fibres and ground substance were often found in the region around the anterior end of the larva. Living larvae were never encapsulated by fibroblasts and fibrous tissue. However, dead larvae were found within nodules which had the typical structure of a foreign body granuloma, with numerous foreign body giant cells engulfing the larval cuticle and necrotic tissue, surrounded by fibroblasts and concentric layers of collagen fibres infiltrated with eosinophils.

The most striking pathological changes were found in the tracks left by the migrating larvae (Figures 2 and 3). Dense accumulations of eosinophils and lymphocytes with some plasma cells surrounded areas where the connective tissue had been dissolved and replaced by an acidophilic exudate

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FIGURE 1. Damage to the oesophageal submucosa around migrating larva of *Hypoderma lineatum*. The connective tissue is oedematous and mildly infiltrated with eosinophils particularly around the blood vessels. Areas of haemorrhage and collagen dissolution surround the larval cuticle. Zenker's fixation, Masson's trichrome stain, × 150.

FIGURE 2. Microscopic changes in oesophageal submucosa behind migrating larva of *H. lineatum*. Almost complete dissolution of the collagenous connective tissue and replacement by a fibrinous, gelatinous exudate moderately infiltrated with lymphocytes and eosinophils. Zenker's fixation, Masson's trichrome stain, × 120.

FIGURE 3. An accumulation of lymphocytes and eosinophils in the oesophageal submucosa around migrating larva of *H. lineatum*. Zenker's fixation, Masson's trichrome, × 200.

FIGURE 4. Channel in the epidural fat from which the larva of *H. bovis* has been removed. There is little inflammatory reaction immediately surrounding the larval cuticle. Zenker's fixation, H. & E., × 120.

FIGURE 5. Microscopic changes in the epidural fatty connective tissue caused by the migrating *H. bovis* larva. Zenker's fixation, Masson's trichrome, × 200.

FIGURE 6. Damage to the periosteum and bone caused by the migrating larva of *H. bovis* in the epidural fatty connective tissue. Zenker's fixation, H. & E., × 120.

FIGURE 7. Degeneration of the epidural fat with the formation of pseudocystic areas filled with a fibrinous material containing a few lymphocytes and surrounded by a zone of fat necrosis, inflammatory cells and giant cells.

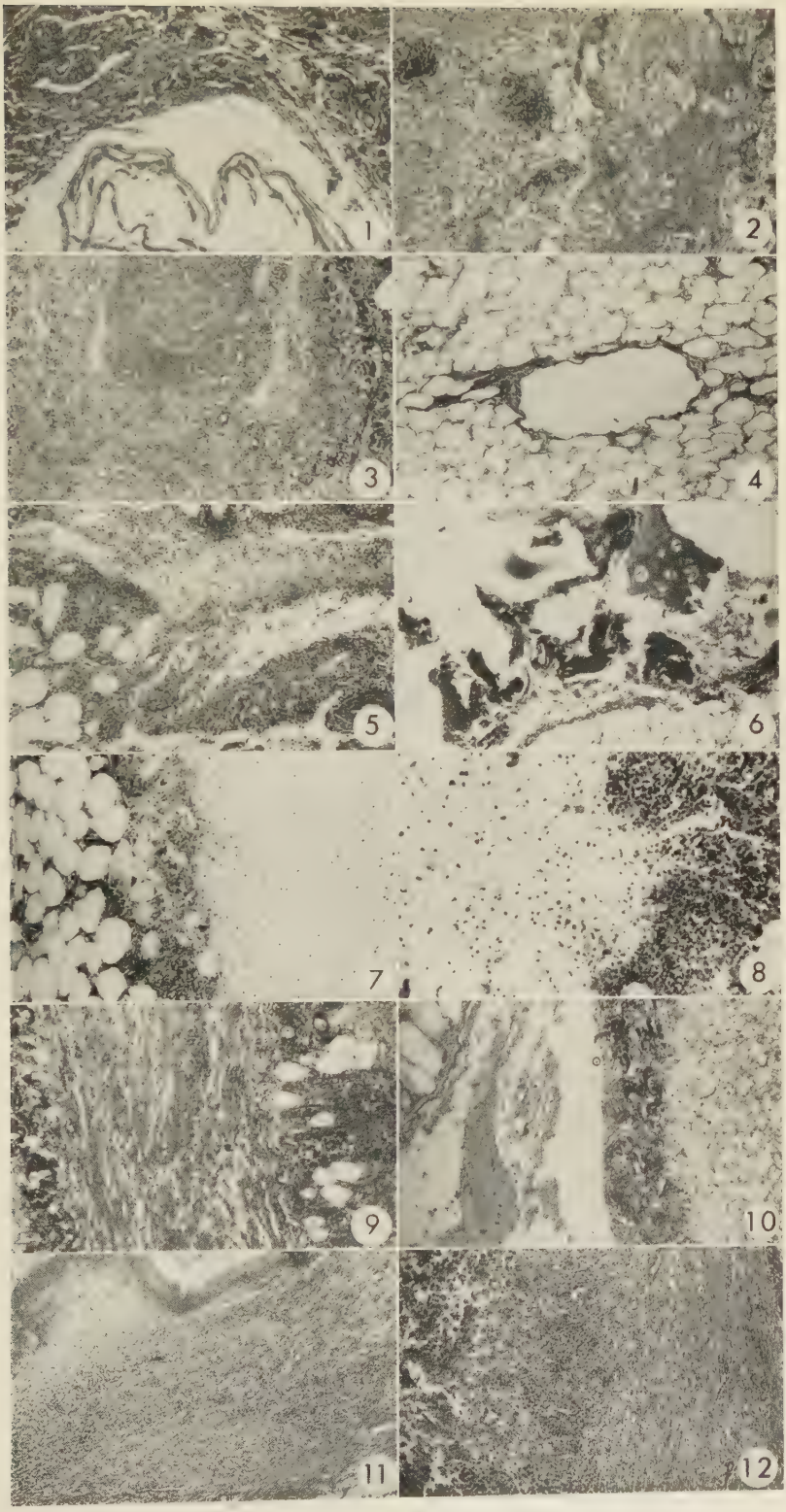
FIGURE 8. Pseudocystic areas in the epidural fat behind the migrating larva of *H. bovis*. A gelatinous, fibrinous material containing erythrocytes, lymphocytes, eosinophils and a few neutrophils. Zenker's fixation, Phloxine-Azure II-Methylene Blue, × 500.

FIGURE 9. Microscopic changes in the connective tissue of "licked beef" caused by the migrating larvae of *H. bovis* and *H. lineatum*. Dissolution of collagen fibres, fat and ground substance and replacement by a greenish-red gelatinous exudate rich in fibrin and infiltrated with eosinophils and lymphocytes. Zenker's fixation, Masson's trichrome stain, × 200.

FIGURE 10. Complete dissolution of the connective tissue surrounding the back muscles caused by the migrating larva. Zenker's fixation, Masson's trichrome stain, × 200.

FIGURE 11. Transverse section through the warble showing the granulation tissue and connective tissue capsule. Zenker's fixation, Masson's trichrome stain, × 120.

FIGURE 12. Section through the warble showing the infiltration with large monocytes and eosinophils and the exudate in the warble cavity. Zenker's fixation, Masson's trichrome stain, × 150.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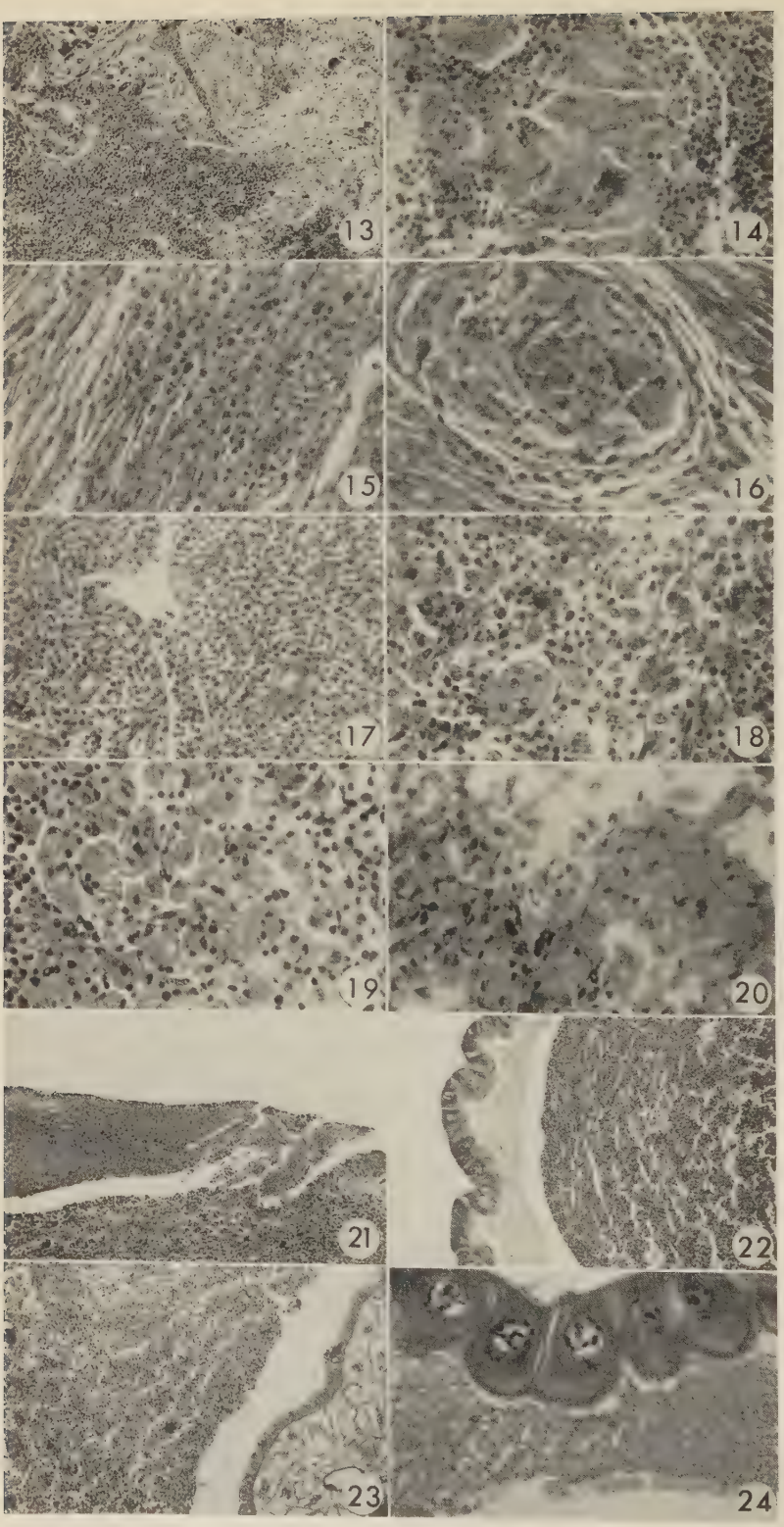
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rich in fibrin and containing fragments of partially dissolved collagen fibres, cellular debris, pycnotic nuclei, as well as a few eosinophils and lymphocytes. The adventitia of the blood vessels were oedematous and infiltrated by eosinophils. Occasionally, areas of haemorrhage into the larval tracks were seen. It was unlikely that the areas of haemorrhage were produced during the stripping of the eosophageal muscular coat. Careful dissection of the muscular coat from the embedded larvae caused very little haemorrhage and the tissue was rinsed before fixation. The lesions left by migrating larvae healed by organization, forming a dense fibrous tissue strand within the submucosa.

The midguts of the migrating larvae were greatly dilated and filled with a translucent, yellowish, homogenous, highly viscous material which stained with acid dyes and was strongly PAS-positive. Pieces of connective tissue, tissue cells, or erythrocytes were never found in the larval midgut. It is thought that the migrating larvae feed on the dissolved products of collagen fibres and ground substance produced by enzymes poured into the tissues by the larvae. The lesions immediately surrounding the anterior end of migrating larvae indicate that the cephalopharyngeal apparatus and mandibles are of minor importance in dissecting the connective tissue. The pathological picture is one of enzymic dissolution and pseudocyst formation with a surrounding infiltration of eosinophils and lymphocytes, rather than a tearing and fragmentation of the connective tissue by mechanical means. A conspicuous feature of the tissue reaction was the absence of neutrophils and any sign of suppurative inflammation.

FIGURE 13. Moulted cuticle of a second-instar larvae in the connective tissue wall of the warble, surrounded by foreign body giant cells and eosinophils. Zenker's fixation, Masson's trichrome stain, × 200.

FIGURE 14. Foreign body giant cells within the granulation tissue of the inner layers of the warble and an area of haemorrhage. Zenker's fixation, Masson's trichrome stain, × 900.

FIGURE 15. Eosinophil and plasma cell infiltration between the collagen fibres in the wall of the warble. Zenker's fixation, Masson's trichrome stain, × 900.

FIGURE 16. A group of giant cells surrounded by fibroblasts and concentric layers of collagenous connective tissue in the wall of the warble. Zenker's fixation, Masson's trichrome stain, × 900.

FIGURE 17. Capillaries filled with eosinophil polymorphs within the granulation tissue in the inner layers of the warble. Zenker's fixation, Masson's trichrome stain, × 900.

FIGURE 18. Monocytes, giant plasma cell, eosinophils and fibroblasts surrounding a dead larva. Zenker's fixation, Masson's trichrome stain, × 100.

FIGURES 19 and 20. Phagocytic monocytes replacing eosinophils in the granulation tissue and warble cavity after the larva has dropped from the back. Zenker's fixation, Masson's trichrome stain, × 1000.

FIGURE 21. Ingrowth of epidermis over the granulation tissue of a vacated warble. Zenker's fixation, Masson's trichrome stain, × 150.

FIGURE 22. Transverse section through the midgut of a larva of *H. bovis* showing the exudate from the warble cavity (in the process of digestion) surrounded by a delicate peritrophic membrane. Zenker's fixation, H. & E., × 200.

FIGURE 23. Further stage in the digestion of the pus cells ingested by the larva. Zenker's fixation, H. & E., × 200.

FIGURE 24. Cellular detail of midgut cells of the larva showing the well-defined striated border against a layer of digested pus cells. Zenker's fixation, H. & E., × 900.

Spinal Canal Stage of H. bovis.

The larvae of *H. bovis* were frequently found embedded in the epidural fat in the epidural space between the dura mater and the periosteum within the spinal canal. Occasionally, larvae were found within the fascia separating the multifidus dorsi muscle dorsally and the quadratus lumborum muscle ventrally. Greenish tracks left by the larvae were readily seen on dissection around the intervertebral foramina and the connective tissue between the deep muscles of the back. Most of the larvae and tracks were found at spinal levels between the eighth thoracic and first lumbar vertebrae. Larval tracks were also found in superficial and deep lumbodorsal fascia. No larvae were found within the epineurium or against the perineurium of spinal nerves. However, larvae were found in the same fascial planes through which the spinal nerves and their branches emerged from the spinal canal.

Larvae within the epidural fat were in small pseudocystic areas surrounded by a yellowish, translucent, viscous fluid (Figure 4). The inner lining of the pseudocyst was green and extended behind the larva and, occasionally, was seen going out through the intervertebral foramina, close to the spinal nerves thus indicating the path of migration of the larva. In the epidural fat the cells had liquefied and degenerated. The areas of liquefaction consisted of a delicate fibrin reticulum containing small collections of pycnotic fat cell nuclei and an occasional eosinophil, neutrophil and lymphocyte. Some specimens showed erythrocytes within the cystic areas. The erythrocytes originated from small blood-vessels that had been destroyed within the epidural fat. The liquefied areas were lined by a zone of fat necrosis characterized by degenerated fat cells, replacement by giant cell systems, and, in addition, dense infiltration with eosinophils of which many were immature forms (Figures 5, 7, and 8). The greenish colour of the larval tracks, apparent on gross examination, was due to the dense accumulations of eosinophils lining the pseudocystic areas and tracks.

Frequently, the inflammation in the epidural fat extended to the periosteum and bone, producing a localized area of periosteitis and osteomyelitis (Figure 6). Occasionally, the epineurium was involved and the perineurium became oedematous and surrounded by eosinophils. In one instance, a larva was found to have penetrated the dura and was lying within the subdural space, producing a small area of meningitis. Dead larvae within the epidural fatty connective tissue were occasionally found. Such larvae were encapsulated with fibrous tissue and the larval material was dissolved by phagocytic monocytes and giant cells and replaced with a caseous material surrounded by chronic inflammatory cells.

The Nature of "Butcher's Jelly"

When the hide was stripped from cattle, the warbles containing larvae alive or dead were removed with it. Frequently, areas of connective tissue left on the carcass at the site of the warbles showed masses of a greenish-yellow gelatinous material. In heavily infested cattle, this material was extensive and voluminous and tracks extended from it along the surface of the superficial muscles of the back. This gelatinous material is called

"butcher's jelly" and the damaged muscle is either discarded or cleaned and down-graded by abattoir inspectors. The material is only found in collagenous or fatty connective tissue and does not extend into muscle. The term "licked beef" is one that was applied by cattlemen and butchers to the traumatized tissue caused by the constant licking of the warbles by the tongue in heavily infested animals. The gelatinous material, however, was frequently found in localized collections beneath warbles in cattle with no sign of external trauma.

Microscopic sections of this "jelly" showed extensive areas where the regular layers of collagenous connective tissue had been completely dissolved and replaced by a fibrinous exudate containing pycnotic fibrocyte nuclei, thin strands of incompletely-dissolved collagen fibres, a few neutrophils, eosinophils and lymphocytes. Surrounding this material was a zone of disorganized connective tissue infiltrated mainly with lymphocytes and eosinophils, small areas of fat necrosis and giant cells, and areas of haemorrhage (Figures 9 and 10). The muscle tissue was unaffected except for a mild oedema in cases where the liquefaction extended as far as the epimysium.

The Warble

The second and third instars do not migrate and are found enclosed, from as early as February to as late as August in Ontario latitudes, in a fibrous tissue capsule producing the familiar swellings known as "warbles" on the backs of cattle. In February, the warbles were found to contain second-instar larvae. A few first-instar larvae that had not perforated the hide were observed surrounded by a small mass of the familiar gelatinous material of the same histological structure as described above. These changes undoubtedly are produced by enzymes produced by the larvae. There was always an associated oedema in the adjacent connective tissue, although the extent of this oedema was very variable.

Once the hide was perforated, the larvae reorientated so that the posterior spiracles faced the opening through the hide. The larvae were now relatively stationary. This, together with the strong foreign-body reaction incited by the protein of the moulted cuticle and excreta of the larvae, probably incited the characteristic cellular infiltration and encapsulation of the larvae by fibrous tissue. Consequently, the warble can be regarded as a large foreign-body granuloma.

Microscopically, warbles containing living larvae showed a mixed picture of chronic granulomatous and chronic non-suppurative inflammation. Only rarely were areas of suppurative inflammation found. The cavity of the warbles contained an exudate consisting of dense collections of necrotic cells and nuclear debris derived from the inner layers of the warble and, in addition, fibrin strands, large numbers of eosinophils and large mononuclear phagocytes, as well as a few lymphocytes, plasma cells, and erythrocytes (Figures 12 and 13). Smears of the exudate stained with Gram's showed no pyogenic organisms but a culture was not attempted. A conspicuous feature was the absence of neutrophils, as first pointed out by Simmons (39). Consequently, the exudate should not in general be termed purulent. However, a few warbles, particularly from western

cattle, showed areas of suppurative inflammation, with a frankly purulent exudate and an extensive area of cellulitis in which the neutrophil polymorph was the predominant inflammatory cell. This was in all probability a result of secondary invasion by pyogenic bacteria through the warble opening. Jettmar (15) has demonstrated an anti-bacterial material in the midgut of larvae. Although a careful bacteriological study is indicated, the type of exudate and cellular infiltration most commonly seen was usually not that of pyogenic inflammation.

Sections through the midgut of larvae within the warbles showed that they fed on the exudate in the warble cavity (Figures 22, 23 and 24). The midgut was packed with inflammatory cells, mainly eosinophils enclosed in a delicate peritrophic sac. The gut contents in the posterior part of the midgut (Figure 23) showed more complete digestion of the ingested cells than in the anterior part.

The histology of the base of the warble surrounding the cephalic end of the larva differed from that part of the warble which surrounded the middle and posterior segments of the larva. The larva completely filled the warble except at the cephalic end where the more mobile segments were embedded in a pocket of exudate. Here, there was no well-defined layer of granulation tissue. The inner layers of the warble consisted almost entirely of collections of large mononuclear cells, eosinophils and plasma cells (Figures 12, 18 and 19). These cells were replaced in deeper layers of the warble by eosinophils and then fibroblasts and collagen fibres, and finally, a thick zone of regular concentric layers of dense collagenous tissue, infiltrated by pockets of eosinophils (Figures 12 and 15). Capillaries were found penetrating to the innermost layers of the warbles (Figures 12 and 17). Small areas of haemorrhage were often found in the inner layers of the warble and within the warble cavity (Figure 14). Gross haemorrhage into the warble cavity was observed infrequently and was usually the result of trauma. The capillaries in the inner zones contained large numbers of immature eosinophil polymorphs (Figure 17). Contrary to general belief, the inner layers of the warble were richly supplied with blood vessels typical of granulation tissue.

In contrast, the sides of the warble cavity were lined by a well-defined, strongly acidophil layer of fibrin mesh, invaded by many fibroblasts (Figure 11). This layer of granulation tissue was 0.5 to 1.0 mm. thick in some areas. Eosinophils and lymphocytes infiltrated the fibrin deposits and newly-formed collagen fibres but were less abundant in the dense collagenous outer zones of the warble. Many newly-formed capillaries were present, with their endothelial sprouts extending to the surface of the granulation tissue. In the inner layers, many undifferentiated or blast forms of the eosinophil and lymphocyte series were found, usually surrounding blood-vessels (Figure 17). The larval cuticle was against the layers of granulation tissue with only a little exudate between. The thickness of the granulation appeared to be related to larval movements which would repeatedly interrupt the healing process and damage the newly formed capillaries, thus leading to the deposition of more fibrin and collagen.

The moulted cuticles of the first- and second-instar larvae were found engulfed by giant cells of the Langhan's type (Figures 13 and 14). Giant

cell systems and tubercle-like follicles with epitheloid cells were frequently found in the outer layers of the warble (Figure 16) and within the granulation tissue. These giant cells formed not only around moulted cuticle, but also around necrotic cellular debris and larval excrement. They were surrounded by eosinophils and various types of histiocytes in different stages of metamorphosis to giant cells.

After the larva had emerged from the warble, the cavity filled with large mononuclear phagocytes (Figure 20). Provided the cavity was not secondarily infected, a tongue of epidermis grew into the warble cavity (Figure 21). The base and sides of the cavity healed by organization, leaving an area of scar tissue which contracted, making a permanent weak spot in the hide. Embedded within the scar tissue were small foreign body granulomas around pieces of cuticle and larval debris. These remained beneath the skin for many months.

Dead larvae were often found in warbles. In this circumstance, the entire cavity was lined with granulation tissue. Large mononuclears and giant cells engulfed and dissolved the cuticle protein and autolysing larval tissues. The cavity of the warble now filled with a cheesy material exhibiting little cellular detail. The fibrous tissue capsule was much thinner around dead than around living larvae. The movement of larvae within the warble appears to stimulate a more profuse deposition of fibrous tissue.

DISCUSSION AND CONCLUSIONS

The tissue reaction provoked by the oesophageal stage of the migrating larvae appears to be caused by the products of enzymic dissolution of ground substance, collagen, and fat. The breakdown products of the host tissues together with the larval secretions cause areas of necrosis of the connective tissue and incite a surrounding inflammatory reaction in which eosinophils and lymphocytes are the predominating cells. Similarly, the observations on the superficial fascia of the back strongly suggest that the collagen fibres, ground substance and fat cells in this region are also dissolved enzymically by secretions of the larvae prior to penetration through the hide. Beneath the skin, the larvae moult to the second instar which immediately becomes encapsulated by circumferential layers of maturing fibrous tissue to form the warble. Enzymes secreted by the larvae before this stage and during perforation of the hide may continue to dissolve the loose connective tissue and, in heavily infested animals, produce large quantities of the gelatinous material known as "butcher's jelly". The products of connective tissue degeneration form pseudocystic areas separated from the normal areolar connective tissue by a zone of chronic non-suppurative inflammation.

The hypodermal stage of the larva provokes a reaction in the tissue of the host similar to that of any foreign tissue. The histological constitution of the warble is that of a granuloma with a well-defined fibrous tissue capsule, endothelial proliferation, formation of giant cell systems, swelling of the vascular endothelium and a marked eosinophilia and lymphocytic reaction. These changes are probably incited by the combined

action of larval secretions, enzymes, and debris, and accentuated by mechanical irritation. The inner layers of the warble are highly vascular and small haemorrhages are common around the larvae.

The routes taken by warbly-fly larvae during their extensive migration through the host tissues are incompletely known. Lapage (20) and Scharff (36) have reviewed the various sites in cattle where larvae have been found. The observations of the larval tracks and position of the larvae in cattle in the present study, together with those of other authors, have led to some suggestions on the route of migration. It is realized that there are still gaps in our knowledge of the complete migration route; however, larvae have been found in a sufficient number of sites for a tentative migration route to be outlined.

First-instar larvae are almost always found in connective tissue. They have been reported in the superficial connective tissue of the hind legs, thighs, loins, flanks, abdomen, back, shoulders, dewlap, neck, forelegs, the loose connective tissue of tendons, the fascia of the superficial and deep back muscles, the epidural fatty connective tissue, the epineurium of the spinal nerves, the pretracheal and peritracheal fascia, the cervical muscle fascia, the oesophageal adventitia, submucosa and lamina propria, the endothoracic fascia, the pericardium, the subperitoneal connective tissue, beneath the capsules of the spleen and liver, the subserosal connective tissue and submucosa of the rumen and intestines, the perirenal connective tissue, the subperitoneal and subpleural connective tissue of the diaphragm, and the connective tissue of the intercostal muscles (2, 3, 4, 8, 9, 10, 11, 12, 13, 24, 28, 33, 35, 41, 42). Larvae are not found within muscular tissue and do not penetrate the striated muscle of the tunica muscularis of the oesophagus except at the junction of the oesophagus and rumen and then only in the connective tissue between the muscle bundles. Popescu-Baran (34) has reported the penetration of the dura mater by larvae and the development of an extensive myelitis, but this is a rare occurrence.

From the evidence at present available, the routes taken by the larvae appear determined by the anatomical arrangement of the superficial and deep connective tissue planes and their interconnections. It has been known for some time (3, 9, 10, 27) that the first-instar larvae of *H. bovis* are frequently found in the epidural space, in the connective tissue around the lower thoracic and upper lumbar intervertebral foramina and in the fascia of the deep back muscles and only rarely in the oesophagus. On the other hand, *H. lineatum* is found regularly in the oesophageal and surrounding connective tissue and only occasionally in the epidural space. It is suggested that a reason for this difference in migration is the difference between the oviposition sites of the two species (*see* Lapage, 20). *H. lineatum* disturbs the cattle less than *H. bovis* and frequently oviposits when the cattle are recumbent. Its eggs are laid around the heel regions, on the forelegs and dewlap, as well as along the ground line. Ono (31) found in Manchuria that the dewlap and forelegs were the preferred sites of oviposition for *H. lineatum*. *H. bovis*, however, generally excites the cattle, causing them to run (i.e., gad), and frequently deposits eggs on the running animals. The eggs are laid more frequently on the upper parts of the hind legs, rump, and loins. *H. bovis* rarely oviposits on the forelegs

and never on the dewlap. The following tentative description of the route of migration of the two species of warble flies is based on the author's personal observations as well as the numerous reported sites, cited above, where larvae have been found.

If first-instar larvae of *H. bovis*, after penetration of the skin, migrate in the subcutaneous connective tissue of the hind legs dorsally they will reach the plane of the superficial lumbodorsal fascia. Not all larvae need enter the epidural space. Those that do appear to follow the planes of the deep lumbodorsal fascia and reach the epidural space by passing in turn through the connective tissue beneath the longissimus costarum, intertransversales lumborum, multifidus dorsi and quadratus lumborum muscles to enter the intervertebral foramina and the epidural fatty connective tissue. These fascial planes are also the pathway of emergence of the thoracic and lumbar spinal nerves. Some larvae may occasionally reach the retroperitoneal and perirenal connective tissue by following the fascia of the quadratus lumborum muscle. The larvae appear to migrate back to the superficial connective tissue and then to the subcutaneous connective tissue of the back by the same fascial planes.

The first-instar larvae of *H. lineatum*, after penetration of the skin of the forelegs or dewlap, appear to migrate dorsally in the superficial connective tissue until they reach the fascial coverings of the cervical muscles. Following these connective tissue planes, the larvae would soon reach the peritracheal connective tissue and the oesophageal adventitia. From there, the larvae could migrate posteriorly through the thoracic inlet towards the rumen and enter the oesophageal submucosa at the junction of the rumen and oesophagus. It is easy to explain the presence of larvae in the pericardium, endothoracic fascia, subpleural connective tissue and subserosal connective tissue of the stomach, spleen and liver if this migration route is followed. From the oesophagus, the larvae probably follow the connective tissue of the diaphragm to reach the fascial planes of the deep muscles of the back and from there reach the superficial connective tissue. Larvae of *H. lineatum* derived from eggs laid on the hind limbs, flanks, escutcheon or udder probably do not pass through the oesophagus but follow essentially the same route as *H. bovis* and occasionally enter the epidural space. Evidence for this has been given by Ono (31) who demonstrated that larvae of *H. lineatum* transplanted subcutaneously in the loin did not enter the oesophagus, but when transplanted in the dewlap were found in the submucosa and adventitia of the oesophagus. Ono further found larvae in the process of migration to the back at the junction of the oesophagus and diaphragm, and in the connective tissue surrounding the intercostal muscles and muscles of the loin and back. There is no evidence that the larvae of *H. lineatum* enter the oesophageal submucosa by penetrating the oesophageal mucosa subsequent to the eggs being licked off the skin and swallowed by the cattle.

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THE ESTROGEN-LIKE SUBSTANCES IN CERTAIN LEGUMES AND GRASSES

II. THE EFFECT OF STAGE OF MATURITY AND FREQUENCY OF CUTTING ON THE ESTROGENIC ACTIVITY OF SOME FORAGES[1]

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ABSTRACT

The seeding year's growth of alfalfa, ladino clover and birdsfoot trefoil has been shown to be essentially free of estrogenic activity. Uninterrupted second-year growth of alfalfa and ladino clover displays relatively high activity in the spring, little or none in mid-summer, and an intermediate level of activity in the autumn. Sequential cutting leads to a modification of this pattern. The presence of slight activity in birdsfoot trefoil and the absence of activity in orchard grass has been demonstrated.

INTRODUCTION

It is now well established that many of the legumes contain substances which, when consumed by the animal, produce effects on the reproductive system comparable with those induced by animal estrogens and synthetic compounds such as diethylstilbestrol (1, 6, 13) or hexestrol (13). The significance of these substances in livestock production is impossible to evaluate until more information is available on their levels in the various forage species. A study of the literature indicates a difference of opinion on the level of estrogenic activity in both fresh and preserved forages. For example, Dohan *et al.* (7) and Legg *et al.* (9) have reported that the alfalfa which they examined did not possess significant estrogenic activity. On the other hand, Pieterse and Andrew (10) stated that alfalfa contains relatively high levels of estrogenic substances. Other work from the same laboratory (11) suggests that alfalfa silage may possess significantly higher levels of these substances than the green forage from which it was prepared. It was implied that the fermentative processes involved in silage preparation were in some way responsible for the increased activity that was recorded. Initial reports suggested that ladino clover is devoid of estrogenic substances (9, 12). More recently (11) it has been established beyond any doubt that it does contain significant levels of such compounds. In fact, Bickoff *et al.* (3) have shown that one of the active substances in ladino clover is coumestrol. This compound is said to possess 30 times more activity per unit mass than does genestein (4), an estrogenically active material which has been isolated from subterranean clover (5).

Information at present available suggests at least five possible explanations for the variations that have been reported in the levels of these estrogenic substances: (a) the chemical nature and the relative activity of the substances may vary in the different species; (b) the chemical nature of the active substances may undergo a series of sequential changes

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yielding compounds that are more or less estrogenic than those from which each originates; (c) the stability of the active substances during harvesting, extraction, diet mixing, digestion or absorption may differ; (d) inherent variability in the forage species, and (e) seasonally induced changes may arise which are independent of the growth pattern of the plant material. The present work was confined to a study of the last of these possibilities.

MATERIALS AND METHODS

Alfalfa (*Medicago sativa* var. Rhizoma), ladino clover (*Trifolium repens*) and birdsfoot trefoil (*Lotus corniculatus* var. Cascade) were selected as legumes representative of those grown in northern regions. Orchard grass (*Dactylis glomerata* var. Hercules) does not possess estrogenic substances (7, 9) but has been included to ensure that these negative findings did not arise because it was harvested at a time when it was devoid of activity. Plots of these forages were seeded in May 1956 and 1957 to provide the necessary quantities of plant material. The procedures used for harvesting, for the preparation of the plant extracts and for the determination of the level of the estrogenic substances in these extracts have already been described (8).

RESULTS AND DISCUSSION

The estrogenic activity of the seeding year's growth of certain forages in relation to stage of maturity and frequency of cutting

From the results recorded in Table I it is apparent that the first year's growth of these forages does not possess measurable estrogenic activity. No explanation can be offered for this interesting finding.

TABLE 1.—THE ESTROGENIC ACTIVITY OF THE FIRST-YEAR GROWTH OF CERTAIN FORAGES IN RELATION TO THE STAGE OF MATURITY AND THE FREQUENCY OF CUTTING

Forage species	Plot	Cutting	Date of cutting (1957)	Per cent dry matter	Per cent nitrogen (dry wt. basis)	Potency in terms of D.E.S. (mcgm.) per lb. dry matter	
Alfalfa	A	1	15/8	15.9	3.9	0	
		2	25/9	24.9	3.7		5
	B	1	25/9	35.9	1.1	0	
Ladino clover	A	1	15/8	9.6	4.2	0	
		2	25/9	12.9	4.0		0
	B	1	25/9	11.9	3.6	0	
Birdsfoot trefoil	A	1	15/8	13.4	2.6	0	
		2	25/9	12.9	2.9		0
	B	1	25/9	21.0	1.7	0	
Orchard grass	A	1	15/8	—	2.1	0	
		2	25/9	31.9	1.6		0
	B	1	25/9	33.1	1.5	0	

TABLE 2.—THE ESTROGENIC ACTIVITY OF THE SECOND-YEAR GROWTH OF CERTAIN FORAGES IN RELATION TO STAGE OF MATURITY

Forage species	Date of cutting (1957)	Stage of maturity	Per cent dry matter	Per cent nitrogen (dry wt. basis)	Potency in terms of D.E.S. (mcgm.) per lb. dry matter
Alfalfa	3/5	Vegetative	15.2	3.5	55
	3/6	Pre-bloom	25.2	3.0	12
	2/7	Full bloom	34.0	2.2	4
	1/8	Early seed	29.9	2.6	21
	3/9	Mid. seed	36.4	1.3	17
Ladino clover	3/5	Vegetative	12.3	4.3	57
	3/6	Mid. bloom	16.0	3.7	9
	2/7	Full bloom	14.6	3.3	5
	1/8	Early seed	26.4	3.3	20
	3/9	Mid. seed	40.9	3.0	20
Birdsfoot trefoil	3/5	Vegetative	12.3	4.0	22
	3/6	Early bloom	22.5	2.8	0
	2/7	Late first bloom	26.5	3.0	0
	1/8	Late second bloom	32.4	2.6	0
	3/9	Mid. seed	38.6	1.8	4
Orchard grass	3/5	Vegetative	18.4	1.9	5
	3/6	Mid. bloom	26.4	1.8	0
	2/7	Full bloom	36.1	1.8	0
	1/8	Mid. seed	40.2	1.0	0
	3/9	Late seed	54.1	0.9	3

The trace of activity in the second cutting of alfalfa harvested on September 25 is of questionable significance because it is at the lower limits of the dose response curve (8). From this evidence it would appear that there would be no hazard associated with the grazing of breeding animals on first-year stands of these legumes in the temperate latitudes.

The estrogenic activity of the second-year growth of certain forages in relation to stage of maturity

The results given in Table 2 show that, in general, the estrogenic activity of the second-year growth of the legumes is relatively high during the early phases of vegetative growth. The activity declines to insignificant levels at the full bloom stage and this is followed by a detectable increase in the early and late seed stages. This latter increase does not occur in birdsfoot trefoil. It is not known whether this increase is associated with the development of seed by the plant or with a change in its vegetative parts. The changes in the estrogenic activity noted do not appear to be associated with changes in the percentage of dry matter or nitrogen in the forages. Orchard grass has again (7, 9) been found to be essentially devoid of activity. The demonstration of reproductive failure in the ovine grazing on subterranean clover by Bennetts *et al.* (2) suggests that caution is necessary if breeding animals are to be pastured on the early spring growth of alfalfa and ladino clover in this latitude.

TABLE 3.—THE ESTROGENIC ACTIVITY OF THE SECOND-YEAR GROWTH OF CERTAIN FORAGES IN RELATION TO FREQUENCY OF CUTTING

Forage species	Cutting	May				June				July				August				September			
		DES	DM	N	SM	DES	DM	N	SM	DES	DM	N	SM	DES	DM	N	SM	DES	DM	N	SM
Alfalfa	1	55	15.5	3.5	V	12	25.2	3.0	PB	4	34	2.2	FB	21	29.9	2.6	ES	17	36.4	1.3	MS
	2					14	18.8	3.5	V	0	20	3.5	V	2	18.2	4.2	V	3	23.1	3.1	V
	3									6	23.8	3.6	V	18	21.8	4.1	V	1	23.4	3.6	V
	4													43	20.5	3.0	V				
Ladino clover	1	57	12.3	4.3	V	9	16.0	3.7	FB	5	14.6	3.3	FB	20	26.4	3.3	ES	20	40.9	3.0	MS
	2					10	15.8	4.2	FB	24	21.7	3.7	FB	11	19.8	3.7	PB				
	3									15	20.3	3.6	FB	42	18.8	4.1	MB				
														30	26.6	4.1	MS				
Birdsfoot trefoil	1	22	12.3	4.0	V	0	22.5	2.8	FB	0	26.5	3.0	LFB	0	32.4	2.6	LSB	0	38.6	1.8	MS
	2					0	15.9	4.4	EB	0	18.1	3.4	FB	0	16.6	4.4	FB	0	22.3	2.8	V
	3									0	17.7	3.2	V	0	17.8	4.2	EB	0	21.4	3.2	V
	4													0	16.1	4.2	EB	0	30.6	2.7	V
	5																	0	32.4	2.8	V
Orchard grass	1	0	18.4	1.9	V	0	26.4	1.8	MB	0	36.1	1.8	FB	0	40.2	1.0	MS	0	54.1	0.9	LS
	2					0	19.6	2.2	V	0	28.1	1.2	V	0	23.5	1.3	V				
	3									0	28.3	1.3	V								

DES—Potency in terms of D.E.S. (mcgm.) per lb. dry matter
DM—Per cent dry matter
N—Per cent nitrogen (dry weight basis)
SM—Stage of maturity
V—Vegetative
PB—Pre-bloom
EB—Early bloom
MB—Mid-bloom
FB—Full bloom
LFB—Late first bloom
LSB—Late second bloom
ES—Early seed
MS—Mid-seed
LS—Late seed

The estrogenic activity of second-year growth of certain forages in relation to frequency of cutting

When the legumes are allowed to follow their normal annual growth pattern without interference by grazing or harvesting, their estrogenic activity is relatively high in the spring, declines to negligible levels by mid-summer and then again increases to an intermediate level of activity in early autumn (Table 2). Since it is customary in practice to make several cuttings when legumes and grasses are harvested for forage, and in an attempt to further elucidate the apparent seasonal change in activity discussed above, representative sequential cuttings were made from certain of the experimental plots. The estrogenic activity, nitrogen and dry matter values of these cuttings are given in Table 3.

In the case of alfalfa, the time at which the first cutting is made appears to influence the estrogenic activity of subsequent cuttings. For example, when the first cutting is made in May and is followed by cuttings in June, July and August the activity tends to follow closely that of plants allowed to grow without interference. On the other hand, when the first cutting is made in June, July and August subsequent cuttings possess little or no activity. Ladino clover behaves quite differently. Regardless of the date of first cutting the activity pattern follows that displayed by alfalfa and ladino clover when they are allowed to grow uninterruptedly. It is possible that the difference between the two legumes is attributable to the indeterminate growth habit of ladino clover; from mid-summer all growth stages are to be found in each cutting. It will be recalled (Table 2) that the early growth of birdsfoot trefoil taken in May had about half the activity of that of alfalfa or ladino clover sampled at the same time. Later samples of this legume, whether taken from uninterrupted growth or sequential cuttings, were devoid of activity. Sequential cuttings of orchard grass do not possess estrogenic activity. It should be emphasized that the results reported are derived from plant materials grown during a single forage season. The possibility that climatic changes from year to year may influence the growth behaviour of the species studied and indirectly their level of estrogenic substances cannot be excluded on the basis of the present data.

On the basis of the above findings one would not expect any deleterious effects on livestock permitted to graze on the aftermath of these forages.

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THE INFLUENCE OF SOLUBLE AND INSOLUBLE GRIT UPON THE DIGESTIBILITY OF FEED BY THE DOMESTIC FOWL[1]

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ABSTRACT

Two experiments were carried out with growing cockerels to study the effect of soluble and insoluble grits upon digestibility. The criterion employed to evaluate these effects was the apparent digestibility of the individual proximate constituents. Comparisons were made between soluble calcitic limestone, insoluble quartz, silica sand and combinations of these grits.

These trials have demonstrated that grit improves feed digestion, particularly of whole grain feeds. In addition, both types of grit were found to be similar in their ability to initiate and maintain this beneficial response, as long as they remain present as distinct entities in the gizzard. It was observed, however, that calcitic limestone was not retained in the gizzard nearly as long as the insoluble quartz grit and consequently a greater consumption of the former may be expected.

INTRODUCTION

The feeding of grit to poultry is considered to be a wise practice. Attempts to determine the actual value of feeding grit have usually entailed a comparison of the growth, egg production, and feed consumption of one group of birds receiving grit with those of a similarly treated group not having access to this material. Results of many of these trials have indicated that birds fed grit tend to utilize their feed more efficiently. Investigations conducted by Fritz (6) leave little doubt that grit improves the digestibility of feeds and, in addition, this improvement is increased as the degree of coarseness of the feed consumed is increased.

The prevalent opinion is that grit aids the gizzard in grinding coarse feed particles (9). However, Waite (10) and Ewing (4) have suggested that the grinding of food particles is only a secondary function of grit, and that its effect on digestibility is achieved primarily by the separation of feed particles in the gizzard.

If grinding is the sole function of grit, then hardness, durability and insolubility would be the criteria for judging its value. Consequently, it would be expected that hard insoluble grits (i.e., granite, quartz, silica sand) would be superior to the soluble grits (i.e., oyster-shell, marble, calcitic limestone). On the other hand, should the second hypothesis be true, then soluble grits would be expected to provide the same benefits as the insoluble grits, assuming that they retain their identity as distinct particles for a reasonable length of time in the gizzard.

The investigation reported herein was designed to study the effect of soluble and insoluble grits upon digestibility and, by direct comparison, to measure the relative efficiencies of the two grits in this role. The criterion employed to evaluate these efficiencies was the apparent digestibility of the individual proximate constituents.

[1]Contribution from the Division of Animal and Poultry Science, Research Branch, Canada Department of Agriculture.

MATERIALS AND METHODS

In view of the relationship between the physical consistency of a ration and its digestibility, as emphasized by Fritz (5, 6), the effects of various grits were studied upon two types of diets, a mash and grain ration and a whole grain ration. The grits involved in this study included an insoluble quartz grit* and a soluble calcitic crystalline limestone grit**. A washed silica sea sand and a mixture of the insoluble and soluble grits were also tested. New Hampshire × Barred Plymouth Rock cross-bred males approximately 10 weeks of age were used in the investigation. These birds had no previous access to grit. The digestibility of the proximate constituents was determined before and after the feeding of grit, thus allowing each bird to be used as its own control.

In Experiment 1, the effect of limestone grit (5 grams per day) on the digestibility of various ration components was determined, using eight individually caged cockerels. A second group of eight cockerels was used in a similar manner to determine the effect of quartz grit (5 grams per day) on digestibility. In both cases the ration consisted of equal parts of growing mash and whole oats, fed at the rate of 50 grams total feed per bird per day.

In Experiment 2, seven individually caged cockerels were used to determine the effect on digestibility of the limestone grit and of sand fed in successive periods. A second group of seven cockerels was used simultaneously to test quartz grit and a mixture of equal parts of quartz and limestone grits for their respective effects on digestibility. In this experiment the ration consisted of a mixture of equal parts whole oats, whole wheat, and cracked corn, fed at the rate of 60 grams total feed per bird per day.

Upon initiation of Experiment 1 the birds were starved for 24 hours, then fed the treatment ration without grit for a period of 7 days. After 3 days on the treatment ration, individual feces collections were carried out for 4 consecutive days. Following this, the same birds were starved for 24 hours and then fed the test ration with the prescribed grit treatment for 3 days prior to, and including, the second 4-day collection period. In Experiment 2, where a second successive grit treatment was imposed, a similar plan was followed. However, the procedure was modified slightly by separating the collection periods with 1-week intervals in which the birds were allowed unrestricted feeding of a standard growing ration. This was an attempt to offset the possible influence on digestion of prolonged feeding of an inadequate all-grain ration.

In both experiments droppings were collected each morning and dried in an oven at 80° C. Upon termination of a collection period the feces from each bird were mixed, ground and sampled for analysis.

Conventional proximate analyses were conducted according to A.O.A.C. methods (1). Nitrogen in the droppings was partitioned into fecal and urinary nitrogen by the method of Ekman *et al.* (3). Organic matter and nitrogen-free extracts attributable to intestinal and urinary sources were calculated, using the method outlined by Olsson and Kihlén (8). Gross energy of the feed and feces was determined employing the Parr Oxygen bomb calorimeter, from which metabolizable energy values were calculated.

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TABLE 1.—INFLUENCE OF GRIT FEEDING ON THE AVAILABILITY OF DIETARY COMPONENTS[1]

Ration	Treatment			Digestibility of the proximate constituents (%)					Metabolizable energy Cal./lb.
	Type of grit	Grit consumption, gm.		Organic matter	Crude protein	Ether extract	Crude fibre	Nitrogen-free extract	
		Daily	Total						
Experiment I									
Growing mash and whole oats (50:50)	Nil	0	0	74.61	83.18	82.29	18.01	80.75	1316
	Limestone[2]	5	20	75.30	83.48	90.19×°	15.91	80.36	1331
	Nil	0	0	74.58	83.28	82.21	17.50	79.69°	1319
	Quartz[3]	5	20	76.33	84.10°	87.61×	18.01×°	81.57×°	1338
Experiment II									
Whole oats, wheat, and cracked corn	Nil	0	0	82.76	82.79	76.35	23.31	88.79	1412
	Limestone	ad lib.	63	88.16×	89.24×	91.54×	55.99×	90.67×	1570×
	Sand	ad lib.	155	90.25×	91.46×	92.82×	60.23×	92.65×	1592×
	Nil	0	0	81.22	81.71	75.89	24.37	86.46	1405
	Quartz	ad lib.	89	87.20×	88.80×	91.54×	48.24×	90.15×	1532×
	Quartz and limestone (50:50)	ad lib.	63	87.63×	90.39×	92.82×	51.44×	91.01×	1545×

× Probability less than .05 when compared to respective controls by appropriate "t" tests of paired data (Goulden, 2nd ed.).
° Probability less than .05 when compared to respective grit treatment by appropriate "t" tests of unpaired data (Goulden, 2nd ed.).

[1] Values represent averages of 8 birds in Expt. 1, and 7 birds in Expt. 2.
[2] York Marble Products Ltd., Fredericton, N. B.
[3] Dominion Silica Corp., Montreal, Que.

RESULTS AND DISCUSSION

The average coefficients of apparent digestibility and the metabolizable energy values are presented in Table 1. In Experiment 1, the addition of grit to the mash and grain ration had a slightly favourable effect. Significant responses, however, were encountered only in the digestibility of the ether extract fraction under the influence of both types of grit, and in the crude fibre and nitrogen-free extract fractions when insoluble quartz grit was fed. On the other hand, in Experiment 2, when whole grains constituted the entire diet, the addition of any grit material caused a significant increase in the digestibility of every proximate fraction. It is, therefore, apparent that a beneficial response to grit feeding can be secured, although the dimensions of response are contingent upon the coarseness of the ration fed.

In both experiments the increased fat utilization brought about by grit feeding was quite marked. Fritz (5) first observed that the cracking or pre-grinding of corn improved fat digestion and later (6) that grit feeding caused a similar response. The data from this investigation confirm these findings. In spite of the improved fat digestion, the metabolizable energy value of the feed in Experiment 1 was only increased slightly while that in Experiment 2 was increased significantly upon the feeding of grit. This result is undoubtedly due to the greater improvement in digestibility of the various constituents other than fat in the second experiment.

Wheeler (11) and Balloun and Phillips (2) have presented data which indicate that a slightly better feed efficiency is brought about by sand as compared to other insoluble grits. Owing to the slight superiority exhibited by sand in this investigation, as opposed to the hen-sized grits, it is suggested that particle size may play some role in the efficiency of grits in the gizzard.

A comparison of the relative efficiencies of the various grits by appropriate "t" test* revealed differences between grit responses in Experiment 1 only. In this experiment significant differences were observed in the digestibility of the protein, ether extract, crude fibre and nitrogen-free extract fractions. Unfortunately neither grit exhibited a clear-cut superiority in all criteria, since crude protein and crude fibre were improved by quartz grit, whereas the ether extract fraction was influenced to a greater extent by the limestone grit. It must be pointed out that the crude fibre determinations were quite variable and must be viewed accordingly. The nitrogen-free extract fraction for some reason was significantly lower in the control quartz grit ration to begin with; nevertheless the effect of quartz grit on the digestibility of this fraction was significantly greater than that of limestone grit.

It is difficult to determine with any degree of certainty the relative value of the two products. Any attempt to do this must reconcile the fact that significant differences between grits have been shown in Experiment 1 but not in Experiment 2. Undoubtedly Experiment 1 utilizes the more practical feeding program; however, the differences due to grit feeding are small and show no definite trend. In addition, by the very nature of

*Goulden, C. H. 2nd ed. Analysis of unpaired data.

TABLE 2.—WEIGHT OF GRIT IN THE GIZZARD FOLLOWING THE FEEDING OF SOLUBLE AND INSOLUBLE GRITS

Number of days from final grit feeding	Grit treatments			
	5 gm./day for 4 days		Unrestricted for 4 weeks	
	Limestone[1]	Quartz[1]	Limestone[2]	Quartz[2]
	gm.	gm.	gm.	gm.
1	4.0	12.0	3.3	12.7
2	1.7	18.0	1.1	10.8
5	Nil	10.0	0.4	7.5
7	Nil	8.5	0.6	9.0

[1] Two birds sacrificed on each day
[2] Four birds sacrificed on each day

the feeding program of Experiment 2, it would be expected that the grits would exhibit their maximum potentialities under these more severe conditions. This being the case it would appear that the insoluble quartz grit, the calcitic limestone grit, sand, and a combination of quartz and limestone grits are equally effective in improving digestibility.

The wearing qualities of the two grits were tested by the method of MacIntyre and Jenkins (7). By this method 74.7 per cent of the insoluble grit was retained on a 40-mesh screen after grinding, while only 56.9 per cent of the soluble grit was recovered on the same sized screen. Obviously the limestone does not possess the hardness nor the durability of the quartz grit. Moreover, the retention of the quartz grit is considerably greater than that of the limestone. This fact was clearly demonstrated when a number of birds were fed grit, then sacrificed, and their gizzard contents examined (Table 2).

In spite of these physical differences the present biological investigation has shown that the responses attributable to the calcitic grit are equal in magnitude to those of the insoluble grits. This would suggest that the mechanism involved in this response, whether it be grinding or the separation of feed particles, is common to both types of grit under test.

Since grit of any kind exerted a greater influence when the diet consisted of whole grains, as opposed to mash and grain, grinding is definitely involved. While the data of Table 2 show that, by feeding grit daily, the constant presence of some gritty material in the gizzards of the birds in both experiments was ensured, there is no evidence to support or refute the view that the separation of feed particles alone contributes to this response. Obviously the equable response to the soluble and insoluble grits demonstrated herein was due to the presence of specific particles of the grit material in the gizzard. These experiments have, therefore, shown that there is no difference between the effect of the soluble and insoluble grits tested upon digestion in the growing chicken, as long as they remain present as distinct entities in the gizzard.

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STUDIES OF BOLUS AND FEED FORMULATIONS OF TWO SYSTEMIC INSECTICIDES FOR REDUCTION OF CATTLE WARBLE INFESTATIONS, (OESTRIDAE: DIPTERA), IN BRITISH COLUMBIA, 1957-1958[1]

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ABSTRACT

In a series of bolus treatment tests, Trolene at dosages of 75 mg., and 110 mg. ronnel per kgm. of animal weight, and Narlene at 15 mg. nellite per kgm. reduced cattle warble, or larval *Hypoderma* infestations 91.2 per cent, 76.8 to 98.8 per cent, and 99.1 per cent respectively. There were no differences in the reductions produced by Trolene at dosages of 75 and 110 mg. per kgm. Narlene was preferable to Trolene for ease of administration. The labour requirements for treatment of a herd of 816 cattle of all ages and a group of 219 steer calves were 19 man-minutes and 10 man-minutes per animal respectively.

In 25-day feed treatment tests, Trolene at 8 and 10 mg. ronnel per kgm. daily, and Narlene at 1 mg. nellite per kgm. daily reduced larvae 87.5 and 96.8 per cent and 91.0 per cent respectively. Feed treatment eliminated the labour and equipment required for bolus treatment.

In a test of Trolene boluses there was no difference in the 22-week post-treatment gains of treated and untreated yearling steers, even though larvae were reduced 86.5 per cent.

Trolene bolus treatment did not appear to aggravate active coccidiosis epidemics and active coccidiosis did not appear to reduce the efficiency of Trolene treatment.

These tests were co-ordinated with British Columbia range management practices. Although these practices seldom permit treatment until much later in the season than is recommended for systemic insecticides, the test results are fully comparable to those reported from areas where the recommendations can be adhered to.

INTRODUCTION

In 1956 Crenshaw (3) reported that a compound known as Dow ET57[3] produced significant reduction of larval *Hypoderma* if administered orally to cattle as a dosage of 110 mg. per kgm. of animal weight before the larvae reached the hypodermal stage. In 1957 extensive tests of drench and bolus formulations were carried out by Canadian and United States agencies and many reports appeared in the literature. Weintraub, Rich and Thompson (10) found that *H. bovis* (L.) and *H. lineatum* (De Vill.) were equally susceptible but larvae became highly resistant after encystment in the hypodermal stage. They, with Adkins (1), McGregor and Bushland (5) and Raun and Herrick (7) reported larval reductions of 97.5, 87.0, 97.4 and 97.2 per cent respectively. Roth and Eddy (8) reported high larval reductions but believed dosage levels were not yet clearly defined. Radeleff and Woodward (6) found a wide range between minimum toxic dosages and minimum lethal dosages.

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[3] Dow Chemical Company code name for O, O-dimethyl O-2, 4, 5-trichlorophenyl phosphorothioate. Application made for registration of *ronnel* as common name for the chemical, registration in 1957 of Trolene for formulations for veterinary use.

TABLE 1.—TREATMENT METHODS FOR A SERIES OF TESTS OF BOLUS AND FEED FORMULATIONS OF TWO SYSTEMIC INSECTICIDES CONDUCTED IN 1957-1958 FOR REDUCTION OF CATTLE WARBLE INFESTATIONS

Herd location	Test No.	Date of treatment	Weather during treatment	No. of cattle treated	Class of cattle treated	No. of cattle sampled		Class of cattle sampled	Treatment	Date of larval collection
						Treated	Untreated			
Empire Valley, Clinton, B.C.	1	Dec. 12 and 13, 1957	25°F. to 33°F. light snow cover	816	All classes	19	30	Calves	Trolene bolus at 110 mg. ronnel/kg.	weekly
70 Ranch, Heffley Creek, B.C.	2	Jan. 14, 1958	28°F. to 32°F. 6″ snow cover	129	Steer calves	30	28	Steer calves	Trolene bolus at 110 mg. ronnel/kg.	Feb. 26, 1958
Range Experiment Station, Kamloops	3	Nov. 21, 1957	33°F. no snow	24	Yearling steers	24	24	Yearling steers	Trolene bolus at 110 mg. ronnel/kg.	Mar. 20, 1958
Harper Ranch, Kamloops, B.C.	4 (a)	Dec. 17, 1957	30°F. light snow cover	150	Calves	29	30	Calves	Trolene bolus at 75 mg. ronnel/kg.	weekly
	4 (b)		Throughout feeding tests–18°F. to 35°F. snow cover increasing to 6″			29			Trolene bolus at 110 mg. ronnel/kg.	
	4 (c)					30			Narlene bolus at 15 mg. nellite/kg.	
	4 (d)					29			Trolene feed[1] at 10 mg. ronnel/kg. daily for 25 days.	
	4 (e)					29			Narlene feed[1] at 1 mg. nellite/kg. daily for 25 days.	
B.C. Sanatorium, Tranquille, B.C.	5	Dec. 20, 1957	Freezing with increasing snow cover	25	Calves	25	25	Calves	Trolene feed[1] at 8 mg. ronnel/kg. daily for 25 days.	Feb. 27, 1958

[1] 10 per cent active insecticide impregnated into fine wheat bran meal

Late in 1957 a Dow ET57 bolus formulation was registered for distribution under the trade-name "Trolene" with a recommendation that treatment not be made after October 30 in any year. Also in 1957 additional compounds and methods of application were introduced. Of the compounds, one termed Dowco 109* appeared to be very promising. The development of Trolene and Narlene formulations for addition to supplement feed ration appeared to open an important avenue of treatment in many types of cattle management.

The current recommendations for the use of these materials emphasize that they should be applied as soon as possible after the cessation of warble fly activity. However, under the open ranging type of management required on the majority of British Columbia ranches, early treatment is impractical. Proper range utilization and fodder conservation prevents gathering of the herds until early winter or just before the appearance of warble larvae in the hypodermal stage. In addition bolus treatment of large and intractable herds presents many handling problems unknown where closer husbandry is practised. Therefore the tests discussed herein were designed to evaluate both the materials and the application methods in relation to British Columbia range management practices.

MATERIALS AND METHODS

The general treatment methods and the conditions during treatment are summarized in Table 1.

All treatments were either made or commenced on the earliest date the animals were available. In all except Test 3 this was during the annual gathering of the respective herd for calf-weaning. Except in Test 3, the sample and test groups were selected by randomization from larger herd groups. Test 3 comprised a group of 48 yearling steers used in a feed utilization test.

The same scale and scale operator were used in all tests except Test 3. All boluses were administered by the same man. Regurgitated boluses were recovered in half-hourly checks of the post-treatment holding pens.

For the feed-tests the formulated insecticide was blended into a commercial calf supplement ration that contained 14 per cent protein and sufficient molasses to prevent separation of the finer elements of the ration. Blending was on the basis of the weight of the test group at an average intake of 2 pounds of supplement per animal per day. Feeding was by free choice in standard feed troughs.

In Tests 2, 3 and 5 a single collection of warble larvae was made at a time estimated to be that of maximum larval abundance. In Tests 1 and 4 all detectable larvae were collected at weekly intervals from the commencement of the hypodermal stage until, on 2 successive weeks, the number collected from the respective untreated group averaged less than 0.5 larvae per animal.

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* Dow Chemical Company trade-name for O-4 (tertiarybutyl-2-chlorophenyl)-O-methyl-methyl-phosphormidothioate. Suggested trade-name for veterinary formulations Narlene, and suggested common name for the compound *nellite*.

TABLE 2.—PER CENT REDUCTION OF HYPODERMA LARVAE RESULTING FROM TREATMENT OF CATTLE WITH SYSTEMIC INSECTICIDES AS OUTLINED IN TABLE 1

Test No.	Date larvae appeared in hypodermal stage	Group	No. cattle in group	Per cent group infested	Average number of larvae	Range of larvae in group	Per cent larval reduction
1	Jan. 1-7	Untreated	30	93.3	30.2	0–135	
		Treated	19	52.6	7.0	0–60	76.8±13.4*
2	Jan. 14	Untreated	28	85.7	33.3	0–192	
		Treated	30	16.7	0.4	0–5	98.8±3.4
3	Jan. 20	Untreated	24	91.7	31.1	0–118	
		Treated	24	66.7	4.2	0–30	86.5±5.3
4	Jan. 14	Untreated	30	96.7	43.3	0–229	
4 (a)		Treated	29	44.8	3.8	0–29	91.2±5.3
4 (b)		Treated	29	34.5	1.6	0–24	96.3±2.1
4 (c)		Treated	30	26.7	0.4	0–2	99.1±3.8
4 (d)		Treated	29	17.2	0.6	0–7	98.6±1.1
4 (e)		Treated	29	48.2	3.9	0–21	91.0±3.3
5	Jan. 1-7	Untreated	25	36.0	3.5	0–27	
		Treated	25	20.0	0.5	0–5	85.7±6.3

* Confidence limits at the 95 per cent significance level calculated from the standard error of the ratio estimate of the treated and untreated infestations

Larval reductions were calculated by the usual comparison of the number of larvae collected from the respective treated and untreated groups. Significance was calculated by the method of standard error of the ratio estimate (2) and appears in Table 2 as the percentage range that would occur at the 95 per cent significance level.

RESULTS AND DISCUSSION

The larval reduction data for all tests are summarized in Table 2.

Trolene bolus treatments produced larval reductions ranging from 76.8 to 98.8 per cent. These are entirely comparable with those previously cited from the literature. In Test 4 there was no significant difference between the reductions produced by Trolene boluses at dosages of 75 and 110 mg. ronnel per kg. This agrees with the observations of De Foliart *et al.* (4) and Wade and Colby (9). The Narlene bolus treatment produced a reduction comparable to the highest reductions produced by Trolene. Furthermore, because of its smaller size, the Narlene bolus was preferable to the Trolene bolus during administration.

The larval reductions produced by the feed treatments are entirely comparable to those produced by the bolus treatments. Neither the feeding behaviour of the calves nor the distribution of the surviving larvae indicated any unpalatability in the feed. The application of treatment did not disrupt the husbandry routine in either location and no special equipment was required. For these reasons the owners and research personnel considered this method to be the most efficient and suitable where cattle receive supplement rations.

In each of these tests treatment was applied on the earliest date the cattle became available. In Test 2 hypodermal larvae were commencing to appear at the time of treatment and had commenced appearing before

Tests 4 (d), 4 (e) and 5 were completed. Furthermore in each instance the weather conditions were such that spray could not have been applied. Therefore, it would appear that under British Columbia ranch conditions oral treatments are the most suitable of the available methods for warble treatment with the available systemic insecticides.

The loss in boluses by regurgitation was less than 0.5 per cent of the total used in Test 1, less than 1.2 per cent in Test 2, less than 4 per cent in Test 3, and 0 in Test 4. There can be little doubt that this loss is lower than that which occurs from run-off during spray application. Also there can be little doubt that insecticide is more uniformly applied by bolus treatment than by spray.

Transitory toxic symptoms were observed after all bolus treatments. In Test 1 two bulls showed excessive salivation with minor bloat and posterior ataxia for 30 hours after treatment. In Tests 2 and 4 about 20 per cent of treated calves were lethargic with minor bloat and some posterior ataxia for 36 hours. In Test 3 a few animals showed minor bloat and diarrhœa, but because their diet had been changed from dry grass forage to alfalfa hay and silage in the 48 hours previous to treatment, this minor upset could not be directly attributed to the insecticide.

Two calves died shortly after treatment but neither death could be directly attributed to the insecticide. In Test 1 a calf became lethargic 36 hours after treatment and died within the next 12 hours. A necropsy failed to show a specific cause of death although it was suggested that protein shock resulting from degeneration of larvae may have contributed. In Test 2 a calf showed throat swelling and loss of appetite about a week after treatment. Despite veterinary treatment the condition became progressively worse and after 3 weeks the animal was killed. Its pharynx was extremely necrotic with several large perforated fistulas extending into the esophagus. The pathological report* attributed the condition to either mechanical or chemical injury. It is suggested that the origin of these esophageal fistulas may have been degenerating larval concentrations in the esophageal mucosa.

In Test 1, 286 calves were treated at a rate of 1 calf per minute and 530 mature cattle at a rate of 1 per 1.4 minutes. An additional 4 hours were required for manipulation of cattle. Twelve persons were required for continuous, efficient operation; therefore the average time expenditure was 18.6 man-minutes per animal. The average dosage was 2.4 boluses. In Test 2, 129 calves were treated at a rate of 1 per minute with an additional 1 hour required for corral handling. Seven persons were required; thus the time expenditure was 10 man-minutes per calf. The average dosage was 1.25 boluses.

In Test 1 a severe epidemic of coccidiosis, *Eimeria* (Protozoa), occurred a few days after treatment and 6 calves died, including 1 from the treated sample group. The incidence of disease was similar in the untreated sample group and the treated calf herd. In Test 2 an even more severe coccidiosis epidemic was commencing at the time of treatment but there was no apparent post-treatment increase in the incidence of disease in the

* Tissue examined by R. J. Avery, Animal Pathology Laboratory, Canada Department of Agriculture, Vancouver, B.C.

treated steer calves and the untreated heifer portion of the herd became more severely affected than the treated steer portion. Therefore, it would appear that the treatment did not aggravate the coccidiosis.

The high larval recoveries in Test 1 suggested that the coccidiosis affecting the intestinal mucosal linings might have reduced the absorption of insecticide. On February 12, 1958, the recovery from the treated sample calves averaged 4.4 ± 10.0 larvae. On February 14, an additional sample taken at the ranch recovered an average of 2.4 ± 5.1 larvae from 37 calves and an average of 3.5 ± 5.6 larvae from 22 aged cows, an age class that is immune to coccidiosis. On February 24, Test 2 showed a larval reduction of 98.8 per cent in the treated group. These data clearly show that the coccidiosis had not affected the efficiency of the insecticide.

In Test 3 the average weight gain during the 22 weeks following treatment averaged 12.7 and 13.3 per cent of the original weight of the treated and untreated steers respectively. The slight difference of 0.6 per cent in favour of the untreated group was not statistically significant. Weight gains were not correlated with the numbers of larvae infesting the steers.

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THE PROTEIN REQUIREMENTS OF LAYING HENS IN FLOOR PENS AND INDIVIDUAL CAGES

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ABSTRACT

Diets varying in protein content from 10.9 to 16.7 per cent were fed over a 336-day laying period to hens in both floor pens and individual laying cages. All rations were approximately iso-caloric, the mean productive energy value being 825 Calories per pound. Each ration was fed to 200-240 hens in four floor pens and to 90-96 hens in individual cages.

A crude protein level of 13 per cent of the diet proved adequate for egg production, but egg weights and body weights were improved when the dietary protein was increased. Egg shell quality was highest on low protein diets. Interior egg quality was not affected by the protein level in the diet.

On comparable diets, birds in individual laying cages laid fewer eggs and consumed less feed than birds in floor pens. Caged birds were heavier in body weight and laid heavier eggs with thicker shells than their counterparts in floor pens. The criteria employed in the measurement of interior egg quality showed no differences between the cage and pen environments, nor did there appear to be any differences in protein requirements between the two environments.

INTRODUCTION

The increasing use of individual cages for laying hens raises a number of questions regarding the nutritional requirements of birds housed in cages as compared with birds in floor pens. It has been observed for some years that hens in cages are subject to physiological changes and abnormalities not observed in birds housed in floor pens, such as atrophy of the heart, gizzard and liver (7), increased fat in the liver (10), and a condition described as cage layer fatigue (3). In addition there is evidence that hens in cages do not lay as many eggs as hens in floor pens (9, 6).

It has been shown by Gowe (6) that some strains of White Leghorns adapt themselves to cage conditions better than others, and Francis (4) has presented evidence suggesting that there are strain differences in susceptibility to cage layer fatigue. However, whether these strain differences reflect differences in nutritional requirements, and whether the conditions described above can be alleviated or corrected by changes in diet remain open questions.

It is quite possible that caged birds require a more complete diet than hens in floor pens, as suggested by Shaw and Nightall (13). On the other hand, there is very little experimental evidence indicating that their actual requirements are different, although King (8) has suggested that birds in cages require less energy and more protein than birds in floor pens.

This paper describes two experiments designed to compare the protein requirements of White Leghorn hens housed in floor pens and in individual

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TABLE 1—COMPOSITION OF EXPERIMENTAL RATIONS

Ingredients	Experiment 1			Experiment 2		
	Ration number			Ration number		
	1	2	3	4	5	6
	lb.	lb.	lb.	lb.	lb.	lb.
Ground wheat	44·75	42·65	40·50	15·00	44·50	47·40
Ground corn	10·00	9·50	9·00	29·75	20·00	10·00
Ground barley	12·50	11·85	11·25	18·025	13·025	12·475
Ground oats	20·00	19·00	17·50	26·85	20·00	11·50
Soybean oil meal (44%)	1·50	5·75	10·50	—	2·10	8·25
Fish meal	2·00	2·00	2·00	1·00	1·00	1·00
Constant ingredients	9·25	9·25	9·25	9·375	9·375	9·375
Total	100·00	100·00	100·00	100·000	100·000	100·000
Crude protein %[1]	13·2	14·9	16·7	10·9	13·1	15·8
Calories per lb.[2]	825	820	814	856	822	822

Constant ingredients: Ground limestone, 4.1%; alfalfa leaf meal, 2.5%; bone meal, 2.0%; iodized salt, 0.5%; manganese sulphate (65%), 0.025%; fish liver oil (1200 A, 150 D), 0.125%; dry Vit. D_3 to supply 150 I.C.U. of D_3 per lb.; riboflavin, 2 gm. per ton. In addition, choline chloride (25%), 0.10% and vitamin B_{12} (6 mg. per lb.), 0.025% were added to all rations in Experiment 2.

[1] Average values determined from analyses on each batch of feed mixed (N x 6.25)
[2] Calculated from Table 18, Titus (15)

laying cages. The diets employed were approximately iso-caloric, and protein response was measured in terms of egg production, feed efficiency, body weight, egg weight and egg quality.

MATERIALS AND METHODS

Two experiments were conducted in successive years using Single Comb White Leghorn hens of similar breeding in both tests. The chickens were hatched about May 1 of each year and were raised in brooder pens. At about 8 weeks of age the pullets were put on grass range. At 140 days of age, pullets with obvious abnormalities were culled from the flock and the remainder randomized into 12 floor pens, 12′ x 16′ in size, and three triple deck laying battery units, each containing 96 individual cages 12″ x 18″ in size. A minimum 13-hour day was provided for both pens and cages by use of artificial lights.

The ingredients in the all-mash rations fed in both experiments are shown in Table 1. The rations contained sufficient calcium to completely meet requirements for egg production and hence no calcium supplement was fed but insoluble grit was provided. Each batch of feed as mixed was analysed for protein content and, if necessary, adjustments were made to keep it within the desired level (Table 1). The average protein content of the three rations over the entire feeding period was 13.2, 14.9 and 16.7 per cent in Experiment 1, and 10.9, 13.1 and 15.8 in Experiment 2. In Experiment 1 each ration was fed to 330 birds, 60 in each of four pens, and

90 in individual cages. In Experiment 2, each ration was fed to 296 birds, 50 in each of four pens, and 96 in cages. In each test the birds were fed *ad libitum* for a period of 336 consecutive days from date of housing.

Egg production was recorded daily on a pen basis for the birds in the pens and on an individual basis for the birds in the cages. All eggs laid during a 4-day period in January were weighed and the average weight for each treatment calculated for pens and cages. Egg-shell quality was determined by measuring the specific gravity of all eggs laid on one day during the months of February, April, July and August. These same eggs were broken out the following day and scored for interior quality on the

TABLE 2—EGG PRODUCTION[1], FEED CONSUMPTION[1], AND FEED EFFICIENCY OF HENS RECEIVING ISO-CALORIC RATIONS OF DIFFERENT PROTEIN CONTENT

Protein level %		Experiment 1			Experiment 2		
		13·2	14·9	16·7	10·9	13·1	15·8
Floor pens	Eggs/bird (No.)	228	235	228	185	212	228
	Feed/bird (lb.)	103·5	99·1	99·5	102·8	107·2	99·1
	Feed/doz. eggs (lb.)	5·5	5·1	5·2	6·7	6·1	5·2
Laying cages	Eggs/bird (No.)	207	216	209	172	190	201
	Feed/bird (lb.)	92·1	91·5	90·9	88·9	90·2	90·7
	Feed/doz. eggs (lb.)	5·3	5·1	5·2	6·2	5·7	5·4

[1] Calculated on a hen-day basis

TABLE 3—AMINO ACID COMPOSITION OF THE DIETS EXPRESSED AS A PERCENTAGE OF THE LAYING HEN'S REQUIREMENTS[1]

Amino acids required by laying hens	Per cent required[2]	Amino acid content of diets expressed as a percentage of the laying hen's dietary requirement					
		Experiment 1			Experiment 2		
		Ration number			Ration number		
		1	2	3	4	5	6
Glycine	0·18	333	494	666	155	339	594
Arginine	·50	118	134	158	100	112	138
Histidine	·16	168	194	218	143	169	200
Isoleucine	·58	91	103	117	81	90	105
Leucine	·70	125	140	155	131	121	145
Lysine	·50*	86	104	126	66	80	108
Methionine	·28*	93	103	111	82	89	100
Methionine+Cystine	·53	90	94	105	75	83	96
Phenylalanine	·46	128	143	181	108	126	150
Threonine	·35	114	131	151	97	108	137
Tryptophane	·15*	93	107	120	73	87	107
Valine	·56	105	118	130	93	102	119
Crude protein%		13·2	14·9	16·7	10·9	13·1	15·8
Effective protein[3]		11·3	14·0	16·7	7·4	10·4	15·2

[1] Amino acid values calculated from Tables 13a and 13b, Titus (15)
[2] Values from Titus (15) p. 99, with exception of values marked x which are from N.R.C. Publ. 301, 1954
[3] Calculated according to Rosenberg (12), i.e. percentage crude protein x dietary level of the limiting amino acid expressed as a percentage of the hen's requirements

basis of the U.S.D.A. score chart (2). Blood and meat spots were also recorded for these eggs. Feed consumption was recorded at 28-day intervals on a pen basis for the birds in the laying pens and on an individual basis for the birds in the cages. All birds were individually weighed at housing time and at the termination of the experiment. No culling was practised in the pens or cages.

RESULTS AND DISCUSSION

The data in Table 2 show the effect of protein level in the diet on egg production and feed efficiency in the pens and cages. In Experiment 1 egg production did not differ between rations in either the pens or the cages. The birds produced just as many eggs on the low protein diet (13.2 per cent) as on the higher levels of protein (14.9 and 16.7 per cent). In Experiment 2, the birds receiving the ration containing 10.9 per cent of protein laid significantly fewer eggs than did the birds on the 15.8 per cent ration in both pens and cages. Production of the birds receiving 13.1 per cent protein was slightly lower than that of the birds on 15.8 per cent protein, but the differences were non-significant.

These data indicate that 13 per cent protein is sufficient for egg production in pens and cages but that 10.9 per cent is inadequate. This differs from the work of Thornton *et al.* (14) which shows no drop in egg production on reduction of the protein level from 13 to 11 per cent, but agrees with the findings of Miller *et al.* (11) who observed good egg production on protein levels of 12.5 to 13 per cent, but poor egg production on protein levels of 10, 11, and 12 per cent.

The discrepancies in the results of different workers studying protein requirements for laying hens may be due in part to differences in the quality of the proteins provided in the test rations. As an indication of the protein quality in the rations used in the present experiments, the essential amino acid contents were calculated from the amino acid composition of the ingredients using the figures supplied by Titus (15) and are expressed as a percentage of the hen's requirement in Table 3. It will be noted that all rations except one (16.7 per cent protein) appear to be low in one or more amino acids. However, only in the case of the 10.9 per cent protein ration in Experiment 2 were these deficiencies reflected in lower egg production. If the effective protein concept, as described by Rosenberg (12) is applied here, this is the only ration with less than 10 per cent effective protein (Table 3). Thus, if effective rather than crude protein is considered, the results suggest that protein levels as low as 10.4 per cent may be adequate for egg production.

When the production of the birds in the pens and cages is compared (Table 2), it will be noted that on comparable rations the caged birds laid fewer eggs and consumed less feed in all cases. There was no significant or consistent difference in feed per dozen eggs in the two environments. The lower egg production of caged hens observed in these experiments confirms the findings of Lowry *et al.* (9) and Gowe (6), but the reason for this effect of the cage environment on egg production remains obscure.

TABLE 4—BODY WEIGHT, EGG QUALITY AND MORTALITY OF HENS FED ISO-CALORIC RATIONS OF DIFFERENT PROTEIN CONTENT

Protein level %		Experiment 1			Experiment 2		
		13·2	14·9	16·7	10·9	13·1	15·8
Floor pens	Mean body weight						
	Initial (lb.)	3·4	3·3	3·3	2·8	2·8	2·8
	Final (lb.)	4·5	4·6	4·7	4·0	4·2	4·4
	Egg quality						
	Weight (gm.)	54·5	54·9	55·3	53·4	53·4	54·8
	Specific gravity	1·0825	1·0821	1·0813	1·0849	1·0838	1·0825
	U.S.D.A. score	2·80	2·80	2·81	2·89	2·92	2·91
	Blood and meat spots (%)	10·0	7·5	5·0	7·8	7·2	7·5
	Mortality (%)	15·0	17·7	14·6	18·5	17·0	23·5
Laying cages	Mean body weight						
	Initial (lb.)	3·3	3·4	3·4	2·9	2·9	2·9
	Final (lb.)	4·7	4·9	4·8	4·3	4·6	4·9
	Egg quality						
	Weight (gm.)	55·0	55·8	55·9	54·1	55·0	56·5
	Specific gravity	1·0842	1·0842	1·0851	1·0861	1·0851	1·0845
	U.S.D.A. score	2·49	2·76	2·56	2·85	2·83	3·01
	Blood and meat spots (%)	6·1	6·8	9·4	6·2	6·4	7·2
	Mortality (%)	7·8	12·2	7·8	10·4	13·5	12·5

It has been suggested by Miller *et al.* (11) that growing birds coming into lay require more protein than fully grown birds. If this is true, the birds on the high protein diets should have come into lay more rapidly than the birds on the low protein rations. However, when the number of days to 25 per cent production was calculated as a measure of sexual maturity, no differences were observed between any of the protein levels, although in both experiments the birds in the cages reached this level of production 3 days ahead of the birds in the pens.

The protein level of the diet had a considerable influence on final body weight and on egg weight particularly in Experiment 2 (Table 4). It appears that where the protein level was not adequate for the maintenance of body weight, egg weights were also depressed. Egg-shell quality, as measured by specific gravity, tended to be higher on the low protein diets, and again the differences were most marked in Experiment 2, where lower levels of protein were tested.

On comparable diets, the birds in the cages were higher in final body weight, laid heavier eggs, and eggs of higher specific gravity in every case (Table 4). The higher specific gravity of the eggs from birds in cages and on low protein diets may be due in part to the fact that egg production was lower under these conditions. It has been shown by Berg (1) that specific gravity varies with the position of the egg in the clutch, the first and last eggs being higher than the intermediate eggs. It follows that the eggs of high producing birds laying long clutches would tend to have a lower average specific gravity.

Albumin quality as measured by the U.S.D.A. method (2) and the frequency of blood and meat spots did not appear to be influenced by the protein content of the diet. Similarly, location in floor pens or in individual cages had no consistent effect on these criteria. The protein level of the diet had no significant effect on mortality, but mortality in the pens was consistently and considerably higher than in the laying cages.

CONCLUSIONS

1. The results of these experiments indicate that 13 per cent protein in the diet is adequate for egg production in floor pens and individual laying cages.
2. There was no indication of a difference in protein requirement between the cage and floor pen environments.
3. Protein level had no influence on interior egg quality but low protein diets resulted in lighter birds laying lighter eggs of higher specific gravity.
4. Environment had a considerable effect on a number of the criteria measured. The birds in the cages were heavier in body weight, and laid fewer but heavier eggs with better shell quality than the birds in the floor pens.

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ON THE PHOSPHORUS REQUIREMENTS OF CHICKENS FOR EGG PRODUCTION AND HATCHABILITY[1]

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ABSTRACT

An experiment was made in an attempt to determine the availability of the phosphorus in soft phosphate as compared with that in dicalcium phosphate for laying hens fed a diet of natural ingredients. A high efficiency "all-vegetable" diet containing 0.38 per cent total phosphorus and an estimated 0.11 per cent nonphytin phosphorus was fed without added phosphorus and with sufficient dicalcium phosphate or soft phosphate to supply 0.05 and 0.1 per cent phosphorus. The calcium content of all diets was kept constant at 2.4 per cent.

The results indicated that the inclusion of supplementary phosphorus did not improve egg production or feed required per dozen eggs. Differences were not demonstrated in body weight maintenance, egg weight, specific gravity of the eggs, hatchability, or bone ash at the end of a 44-week period of production.

The results suggest the possibility that egg weight loss after 14 days' incubation may have been influenced by supplementary phosphorus. Evidence for this effect was not clear-cut and more work appears desirable in this connection.

It is concluded that it is not possible to assay feed phosphates for laying hens with diets of natural ingredients. It would also appear that a level of 0.38 per cent total phosphorus and 0.11 per cent nonphytin phosphorus is adequate for laying hens.

Supplementation of an "all-vegetable" laying diet with Vigofac did not improve egg production, feed efficiency, egg weight, egg-shell quality or hatchability.

INTRODUCTION

Relatively few papers have appeared in the literature on the phosphorus requirements of laying hens and there is a lack of agreement between the results of various investigators. Messengale and Platt (4) obtained as good production with a level of 0.5 per cent of phosphorus in the diet as with higher levels. Norris and co-workers (8) found 0.5 per cent phosphorus to be insufficient for maintaining normal egg production, while 0.75 per cent was adequate. Miller and Bearse (5) obtained higher production on diets containing approximately 0.8 per cent phosphorus than on diets containing either more or less of this element. Mitchell and McClure (6) calculated that a level of 0.32 per cent phosphorus should be sufficient for 50 per cent production and 0.35 per cent should be adequate for 75 per cent production if 50 per cent of the phosphorus is utilized.

After reviewing the literature Schaible (11) concluded that 0.4 per cent phosphorus was required, but to allow for a margin of safety 0.5 per cent was recommended. Evans and Carver (2) stated that the phosphorus requirement was 0.6 per cent in the presence of 1.5 per cent calcium and 0.8

[1] A preliminary report of this work was presented at the annual meeting of the Poultry Science Association at Cornell University, Ithaca, N.Y., August, 1958.

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per cent in the presence of 2.5 per cent calcium. When 3.0 per cent calcium was included in the diet 0.8 per cent phosphorus was not as satisfactory as 1.0 per cent.

O'Rourke and co-workers (9) found that a semi-purified diet containing a total of 0.19 per cent phosphorus did not support normal egg production and hatchability. Supplementing this basal ration with $CaHPO_4.2H_2O$ to contain 0.30 per cent phosphorus resulted in normal egg production and hatchability being maintained. The basal ration, which proved inadequate, contained 0.12 per cent phytin phosphorus and 0.07 per cent nonphytin phosphorus while the adequate ration contained 0.12 per cent phytin phosphorus and 0.18 per cent nonphytin phosphorus. Phosphorus levels between these extremes were not employed. A review of the literature indicates that this is the first published report where a sufficiently low level of phosphorus was used to affect hatchability. The hatchability of eggs from the pullets fed the basal diet decreased to a low of 20 per cent after 12 weeks on feed. When the phosphorus level in the feed for these birds was increased to 0.30 per cent, the hatchability returned to normal within 3 weeks. The main increase in embryonic mortality due to phosphorus deficiency occurred after the first week of incubation. No specific gross abnormalities were observed which would distinguish the embryos as being phosphorus deficient.

In further work O'Rourke *et al.* (10) found that a practical diet containing 0.35 per cent phosphorus, nearly all from plant sources, although deficient for maximum growth, contained enough phosphorus for normal date of first egg and egg production. Also, the pullets fed the basal ration achieved normal weight at least by 42 weeks of age. These workers also found in three other experiments that no more than the 0.43 per cent phosphorus found in the practical "all vegetable" basal diet was needed to maintain egg production at a normal level. The requirement for phosphorus by the laying pullet could not be determined in their work as the practical diets employed were not sufficiently low in phosphorus to demonstrate a deficiency.

The National Research Council (7) recommended a level of 0.6 per cent total phosphorus for laying and breeding hens. They suggest that a portion of this requirement should be in inorganic form.

The present experiment was designed to determine the availability of the phosphorus in soft phosphate (Sea-Coll) for laying hens. Dicalcium phosphate (Dynafos) was used as a standard of comparison. While the practical basal diet used was shown to be too high in phosphorus to permit such a comparison the results provide further information on the phosphorus requirements for egg production and hatchability.

MATERIALS AND METHODS

Stock and Management

For the experiment each pen of pullets was comprised of four subgroups of 15 birds each of Hyline, Dekalb, Babcock and a Columbian Plymouth Rock x Rhode Island Red cross, for a total of 60 birds per pen. All birds were reared in confinement and fed a high efficiency type diet on

a restricted basis. The starting and growing diets contained about the levels of phosphorus recommended by the National Research Council (7). The laying experiment was begun in December and was conducted in a laying house with a central alley and six pens on either side. Wood shavings were used as litter in the pens.

Diets and Feeding

Duplicate groups (pens) of birds were fed an "all-vegetable" basal diet (Table 1) and this diet supplemented with sufficient dicalcium phosphate or soft phosphate to increase the level of phosphorus by 0.05 and 0.10 per cent. The fermentation product Vigofac, which has been shown to be a source of "fish" factor and "whey" factor activity for chicks (Summers *et al.* 12), was included in the diet for two groups. It was felt that if this product exerted a beneficial effect on egg production it might be useful for future assay work in that it would provide unidentified factors without an appreciable increase in the phosphorus content of the basal diet. The

TABLE 1—COMPOSITION OF BASAL DIET

Ingredient	Per 100 pounds
	pounds
Ground wheat	40·0
Ground yellow corn	31·2
Soybean oil meal (44% protein)	18·0
Animal fat (stabilized)	1·5
Dehydrated alfalfa meal (17% protein)	3·0
Iodized salt (0.015% KI)	0·5
Ground limestone	5·8
	grams
Manganese sulphate (75%)	5·7
Vitamin A suppl. (10,000 I.U./gm.)	5·7
Vitamin D_3 suppl. (1,500 I.C.U./gm.)	28·4
Riboflavin suppl. (1 gm./oz.)	3·5
Procaine penicillin G (feed grade)	0·2
Vitamin B_{12} suppl. (6 mg./lb.)	22·7

TABLE 2—PLAN OF EXPERIMENT

Group No.	Treatment	Calculated phosphorus	
		Total	Inorganic and nonphytin
		%	%
1A* and B*	Basal	0·37	0·11
2A and B	Basal + 0.05% P from dicalcium phosphate	0·42	0·16
3A and B	Basal + 0·1% P from dicalcium phosphate	0·47	0·21
4A and B	Basal + 0·05% P from soft phosphate	0·42	0·16
5A and B	Basal + 0·1% P from soft phosphate	0·47	0·21
6A and B	As 3A and B + 0.15% Vigofac	0·47	0·21

* Duplicate groups of 60 birds were used for each treatment.

phosphate supplements were added to the basal diet at the expense of ground yellow corn and ground limestone in such a manner as to keep the level of calcium constant in all diets as the phosphorus level was increased. Vigofac was added at the rate of 0.15 per cent to replace ground yellow corn. The calculated calcium level for all diets was 2.28 per cent while the protein level of the basal diet was calculated at 16.2 per cent. Thirty per cent of the total phosphorus in plant products and all the phosphorus in the phosphate supplements was assumed to be inorganic and nonphytin in nature. The diets were mixed about twice a month during the experiment and analyses for crude protein, calcium and total phosphorus were made on the basal diet on fourteen occasions. Based on these fourteen mixes the average value for crude protein was 16.6 per cent, that for calcium 2.44 per cent and that for total phosphorus 0.38 per cent. The calculated and determined values for total phosphorus in the basal diet are in excellent agreement. The diet plan of the experiment together with the calculated phosphorus levels are shown in Table 2.

The all-mash experimental diets and water were available at all times, starting when the birds were 22 weeks of age and continuing for a 44-week period of production. No calcium supplement was offered on a free choice basis and hen-size granite grit was fed 1 day per week at the rate of 2 pounds per 100 birds for the first 24 weeks of production. This was reduced to 1 pound per 100 birds per week for the last 20 weeks of production since there was some reluctance on the part of some groups to consume the larger amount.

Response Criteria

Records were kept of total egg production, feed consumption, body weight and mortality. In addition, the birds were trap-nested 3 days per

TABLE 3—EFFECT OF PHOSPHORUS SUPPLEMENTS AND VIGOFAC ON EGG PRODUCTION AND FEED EFFICIENCY

Group No.		Weeks on experiment						
		0 to 4	8 to 12	16 to 20	24 to 28	32 to 36	40 to 44	0 to 44
1	P	41·2	81·9	84·0	82·7	66·0	56·6	72·0
	E	6·52	3·75	3·67	3·67	3·88	5·09	4·10
2	P	46·6	80·9	81·9	79·8	65·7	56·6	71·3
	E	5·89	3·81	3·79	3·76	3·98	5·29	4·17
3	P	42·6	81·5	83·6	81·8	66·1	62·9	72·3
	E	6·43	3·68	3·60	3·63	3·70	4·51	4·00
4	P	44·2	77·8	79·3	77·0	63·9	57·2	69·6
	E	6·13	3·91	3·88	3·93	3·99	5·17	4·24
5	P	45·2	80·2	80·5	78·4	61·3	58·3	69·7
	E	6·08	3·88	3·88	3·86	4·06	5·12	4·25
6	P	42·9	79·8	83·1	79·2	68·8	57·3	71·1
	E	6·38	3·81	3·68	3·80	3·78	5·08	4·13

P—per cent production on a hen day basis
E—pounds of feed per dozen eggs

week in order to obtain egg production data on the four strains and crosses of birds. Egg weight, egg weight loss after 14 days' incubation, and egg specific gravity measurements were made at intervals during the course of the experiment.

Four cockerels were placed in each pen during the 28th week of production and 3 days' eggs were saved for incubation during each of the following 3 months in order to obtain hatchability data. The cockerels were fed the basal diet for 1 week prior to being placed in with the pullets in order to determine their average feed consumption. The feed consumption of the cockerels, so determined, was subtracted from the total feed eaten by each pen in order to continue to determine feed required per dozen eggs as before. The cockerels were rotated weekly.

Three birds of each strain or cross in each pen or a total of 24 birds per treatment were sacrificed at the end of the 44-week period of production. The left tibiae of these birds were removed, cleaned of adhering tissue and the percentage ash determined on the fat-free, dry bones according to the method of the Association of Official Agricultural Chemists (1) for vitamin D assay.

Experimental Design and Analysis of Data

The experiment was conducted in a split-plot design with rations as the main plot, strains as a first split and time in production as a second split. Where appropriate the response data were analysed statistically according to the following plans:

Source of Variation	Degrees of Freedom		
	Egg production	Body weight	Egg weight Egg weight loss Egg specific gravity
Replicates	1	1	1
Diets	5	5	5
Error (a)	5	5	5
Total	11	11	11
Strains	3	3	3
Diets × strains	15	15	15
Error (b)	18	18	18
Total	47	47	47
Time	—	4	2
Time × diets	—	20	10
Time × strains	—	12	6
Time × diets × strains	—	60	30
Error (c)	—	96	48
Total	—	239	143

All statements with respect to statistical significance are made at the 5 per cent probability point.

RESULTS AND DISCUSSION

Egg Production and Feed Efficiency

The effect of the phosphorus supplements and Vigofac on egg production and feed per dozen eggs is shown in Table 3. To conserve space only the results for alternate 4-week periods together with the cumulative egg production and feed efficiency values are presented. It may be noted that all groups laid at a satisfactory rate. The addition of phosphorus from dicalcium phosphate caused little improvement in egg production or feed required per dozen eggs while soft phosphate gave slightly poorer values as compared with the control. Vigofac failed to exert any appreciable influence on egg production or feed efficiency. A statistical analysis indicated that none of the differences in egg production or feed required per dozen eggs, due to dietary treatments, was significant.

The egg production data for individual strains of birds will not be presented at this time. A statistical analysis of these data indicated no significant strain x diet interaction. There were, however, significant differences between the strains.

Live Weight

The birds were weighed individually at the start of the experiment and after 12, 24, 36 and 44 weeks in production. The body weight data are shown in Table 4. The birds in all groups gained in weight until 24 weeks of age, with two strains markedly outgaining the other two (not shown in Table). From 24 to 36 weeks body weights decreased and remained reasonably constant or increased slightly from 36 to 44 weeks in production. From 24 weeks onward the weights of all strains followed parallel courses. The loss in weight corresponded with the period of highest summer temperature.

There was little difference in body weight alteration which could be attributed to the level of phosphorus since the time x diet interaction was not significant. In view of the fact that a relatively high energy basal diet was used it is of interest that all groups lost weight during the summer months. Heuser *et al.* (3) fed purified rations containing 0.25 and 0.5 per

TABLE 4—EFFECT OF PHOSPHORUS SUPPLEMENTS AND VIGOFAC ON BODY WEIGHT

Group No.	Weeks on experiment				
	0	12	24	36	44
	Average body weight				
	grams	grams	grams	grams	grams
1	1709 (120)*	2002 (116)	2048 (115)	1882 (108)	1905 (107)
2	1717 (120)	1997 (118)	2059 (114)	1906 (110)	1939 (110)
3	1705 (120)	1999 (117)	2086 (115)	1927 (113)	1921 (110)
4	1738 (120)	2023 (118)	2103 (111)	1949 (101)	1967 (100)
5	1752 (120)	2021 (119)	2093 (114)	1923 (106)	1974 (105)
6	1700 (120)	2046 (116)	2108 (115)	1962 (110)	1974 (109)

* Brackets indicate number of birds.

TABLE 5—EFFECT OF PHOSPHORUS SUPPLEMENTS AND VIGOFAC ON EGG WEIGHT

Group No.	Weeks on experiment		
	9	22	34
	Average egg weight		
	grams	grams	grams
1	55·3 (280)*	59·7 (290)	60·2 (204)
2	55·8 (253)	60·2 (274)	61·0 (209)
3	54·5 (279)	58·4 (284)	59·3 (211)
4	55·0 (264)	59·5 (266)	59·9 (186)
5	54·4 (286)	59·3 (266)	59·5 (191)
6	54·5 (269)	59·3 (271)	59·7 (223)

* Brackets indicate number of eggs.

TABLE 6—EFFECT OF PHOSPHORUS SUPPLEMENTS AND VIGOFAC ON EGG WEIGHT LOSS

Group No.	Weeks on experiment		
	9	22	34
	Average weight loss[1]		
	%	%	%
1	7·47 (280)*	7·82 (290)	8·21 (204)
2	7·38 (253)	7·68 (274)	7·77 (209)
3	7·41 (279)	7·75 (284)	8·15 (211)
4	7·08 (264)	7·53 (266)	7·85 (177)
5	7·29 (286)	7·61 (266)	7·78 (191)
6	7·43 (269)	7·94 (271)	8·16 (218)

[1] After 14 days' incubation at 99.5°F.
* Brackets indicate number of eggs.

cent inorganic phosphorus to laying hens. The only difference between these two levels was that the lot receiving the 0.25 per cent of inorganic phosphorus showed poorer maintenance of body weight and a lower content of inorganic phosphorus in the blood than the lot receiving the 0.5 per cent level. It would thus appear that phosphorus is necessary for body weight maintenance and, since all of the diets used in our work were lower than 0.25 per cent in inorganic phosphorus, it was perhaps to be expected that they would fail to maintain body weight. Since production characteristics appear to be satisfied with these lower levels of phosphorus it may well be that lowering the dietary phosphorus will prove to be a satisfactory means of preventing laying and breeding birds from becoming overly fat.

It may be noted from Table 4 that the mortality in the experiment was moderate and did not appear to be related to the diets fed. Most of the mortality was due to "pick-outs" or leucosis.

Egg Characteristics

Three days' eggs were employed for measurement of egg weight, and egg weight loss after 14 days' incubation at 99.5°F., on each of three occasions, namely after 9, 22 and 34 weeks' production. The egg weight data are presented in Table 5. The results indicate that the differences in egg weight were small with no consistent trends due to dietary treatments being apparent. The weight of a "large" egg according to Canadian grading regulations is 56.7 grams. It is of interest that all dietary treatments resulted in an average egg weight approaching this value after 9 weeks of production and that the average for each treatment exceeded this weight after 22 weeks of production.

The egg weight loss data are presented in Table 6. As was to be expected egg weight loss increased with time in production. The diet effects appeared to be reasonably consistent with length of time in production. This was borne out by the finding of a non-significant diet x time interaction. Although the egg weight loss with dicalcium phosphate was found to be significantly greater than that with soft phosphate, the responses to phosphorus increments were not parallel in the two cases (average percentage loss for the three time periods: control 7.80, 0.05 per cent dicalcium phosphate 7.60, 0.1 per cent dicalcium phosphate 7.75, 0.05 per cent soft phosphate 7.50, 0.1 per cent soft phosphate 7.55). Addition of 0.1 per cent dicalcium phosphate resulted in about as much loss as no addition while inclusion of 0.05 per cent dicalcium phosphate reduced weight loss by 0.2 percentage points. In the case of soft phosphate both increments of phosphorus reduced egg weight loss and to about the same extent. Since the phosphorus in soft phosphate is lower in availability for the chick than that in dicalcium phosphate (Summers *et al.* 13), it may be that soft phosphate contains a factor other than phosphorus which aids in shell formation. The effect of Vigofac on egg weight loss was not found to be significant (Group 3 vs. Group 6).

The egg weight loss data for the various strains will not be presented in detail. Statistical analysis of these data indicated that the strain x diet interaction was not significant. However, there were significant strain differences in egg weight loss.

TABLE 7—EFFECT OF PHOSPHORUS SUPPLEMENTS AND VIGOFAC ON SPECIFIC GRAVITY OF EGGS

Group No.	Weeks on experiment		
	10	23	35
	Specific gravity	Specific gravity	Specific gravity
1	1·0853 (274)*	1·0827 (282)	1·0791 (199)
2	1·0841 (266)	1·0825 (261)	1·0786 (214)
3	1·0852 (278)	1·0825 (283)	1·0775 (226)
4	1·0841 (257)	1·0816 (261)	1·0771 (182)
5	1·0841 (282)	1·0816 (260)	1·0774 (184)
6	1·0849 (274)	1·0822 (263)	1·0771 (237)

* Brackets indicate number of eggs.

Specific gravity was determined on 3 days' eggs after 10, 23 and 35 weeks' production. The average values for the treatments are presented in Table 7. It is of interest that the birds fed the basal diet without added phosphorus (Group 1) produced eggs which were of slightly higher specific gravity than those of the remaining birds. However, a statistical analysis of the data showed that there were no significant differences due to diets. The specific gravity data for the individual strains will not be presented since the strain x diet interaction was not significant. However, it is of interest that significant differences in specific gravity were found to exist between strains. Comparing the specific gravity data (Table 7) with the egg weight loss data (Table 6) it is obvious that there was little or no correlation between these two measurements.

Hatchability and Fertility

The hatchability and fertility data are presented in Table 8. These data indicate that hatchability was of a relatively high order and did not appear to be greatly influenced by diet. Fertility, on the other hand, was generally low, with the suggestion that the control diet (Group 1) gave poorer fertility than the remaining treatments. This was true in both replicates and in each of the three time periods. It should be borne in mind that these data were obtained after the birds had been in production for 28 weeks. This, coupled with the facts that the environmental temperature was relatively high at the time the hatching studies commenced and the cockerels used had been in service for about a year previous to being used here, might well account for the generally low level of fertility.

A statistical analysis of the hatchability data indicated that there were no significant differences due to diet. The effects of both dicalcium phosphate and soft phosphate on fertility proved to be linear, suggesting that

Table 8—Effect of phosphorus supplements and Vigofac on fertility and hatchability

Criterion	Group No.					
	1	2	3	4	5	6
Eggs set, no.	598	608	649	543	547	627
Infertile, %	33·6	18·6	15·1	24·0	17·1	23·1
Hatchability of fertile eggs, %	91·7	92·7	93·7	90·2	94·3	92·7

Table 9—Effect of phosphorus supplements and Vigofac on bone ash after 44 weeks of production

Criterion	Group No.					
	1	2	3	4	5	6
Bone ash (%)	56·1	58·2	56·6	57·2	57·1	56·1
Number of birds	24	24	24	24	24	24

the basal diet may have been too low in phosphorus for maximum fertility. According to the analysis, Vigofac depressed fertility significantly (Group 3 vs. Group 6).

Bone Ash

The birds used for bone ash determination were taken from the trap-nests and were all in production. It was desired to use birds which had been in production for the longest possible time and to use no birds which were out of production. The bone ash data are shown in Table 9. The differences were small and not consistently related to diet. Statistical analysis of the data indicated that there were no significant differences in bone ash due to dietary treatments. The data for individual strains will not be presented. There was found to be no significant strain x diet interaction.

General

While this experiment was not designed primarily to study phosphorus requirements, the results confirm the findings of O'Rourke and co-workers (10) in indicating that the requirement of laying hens for total phosphorus is less than generally assumed. Based upon the present study it would appear that a diet containing 0.38 per cent total phosphorus and 0.11 per cent nonphytin phosphorus is adequate for egg production. The indication from these data that the lowest level of phosphorus might not be adequate for minimum egg weight loss is not at all conclusive. In any case the difference in this regard due to the addition of phosphorus was very small and of questionable practical significance. Also, the suggestion that the lowest level of phosphorus, while adequate for hatchability, may not be sufficient for maximum fertility, must be interpreted with caution in view of the findings of O'Rourke *et al.* (9). These workers found that a diet containing 0.12 per cent phytin and 0.07 per cent nonphytin phosphorus did not support normal egg production or hatchability. However, this diet apparently supported normal fertility. It would thus appear that egg production and hatchability are more critical than fertility as criteria of phosphorus deficiency. Since infertility in the present study was determined by candling and macroscopic examination of the opened eggs and not by microscopic examination, it is possible that some of the eggs classed as infertile were actually early embryonic deaths. If this were true, it would suggest that the lowest level of phosphorus used was not adequate to support normal hatchability. However, this hypothesis would not be in agreement with the findings of O'Rourke *et al.* (9) who found that with a phosphorus-deficient diet the main increase in embryonic mortality occurred after the first week of incubation. Based on the above considerations it appears possible that the apparent differences in fertility in the present experiment may have been due to chance.

As in the work of O'Rourke *et al.* (10) the phosphorus requirement for egg production could not be determined in this study as the practical diet employed was not sufficiently low in phosphorus to demonstrate a deficiency. In their work oyster shell was offered to the birds on a free choice basis. The present results indicate that essentially similar results can be expected

when the required amount of calcium is included in the diet. In view of the fact that energy level, fibre content and other factors may influence the phosphorus requirement, the low level of phosphorus found to be adequate in this work would not necessarily be sufficient for diets differing markedly in composition from the one used here.

Summers *et al.* (12) found Vigofac to be a source of "fish" factor and "whey" factor activity for growing chicks. The present results would therefore suggest that laying and breeding hens fed an "all-vegetable" diet and maintained on litter do not require a supplementary source of these factors.

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THE EFFECT OF CERTAIN ORAL AND INJECTABLE IRON PREPARATIONS ON THE BLOOD OF BABY PIGS[1]

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ABSTRACT

Haemoglobin levels, haematocrit values and erythrocyte counts were determined at weekly intervals from 3 to 45 days of age for 60 pigs which received iron in the form of: injectable iron-dextran (A); injectable iron-dextran (B); injectable ferric ammonium citrate; oral iron in the form of paste, or sods sprinkled with iron sulphate. The iron-dextran and ferric ammonium citrate compounds were administered at 3 days of age as single injections supplying 100 mgm. of iron and 30 mgm. of ferric ammonium citrate respectively. The paste was administered at 3, 10, 17 and 24 days of age. Sods were fed twice a week during the period of 3 days to 28 days of age.

The sod treatment maintained normal blood values while the iron-dextran compounds and the paste resulted in values somewhat below normal, although visible evidence of anaemia was not apparent. Blood values for the group receiving ferric ammonium citrate were extremely low and two pigs on this treatment died at 42 and 60 days of age. Both exhibited severe anaemia.

Significant differences were obtained in weaning weights. The heaviest pigs were those receiving sods; the lightest pigs those receiving injectable ferric ammonium citrate. The effect of the different treatments on growth was not apparent until after 21 days of age.

INTRODUCTION

It has long been known that mammary transfer of iron is inadequate to meet the requirements of the suckling pig. In order to maintain normal blood values, supplemental iron must be provided. 'Normal' ranges for the different blood constituents in immature pigs, as reported by Coffin (5), are: haemoglobin, 9.0–16.8 gm. per 100 ml; haematocrit, 32–47 per cent; erythrocytes, 5–9 million per cc.; mean corpuscular volume (MCV), 50.0–66.5; mean corpuscular haemoglobin (MCH), 16.2–22.5. Values reported as normal by Dukes (7), Merck (13) and Wintrobe (20), all fall within these ranges.

Sods with or without the addition of an iron sulphate solution fed at regular intervals or oral iron in one of several forms have been the standard methods of providing additional dietary iron. Each of these methods requires that the iron supplement be supplied at periodic intervals throughout the suckling period. This is laborious and often neglected with the consequence that many baby pigs suffer from anaemia that may vary from mild to severe.

Recent advances in swine nutrition include the development of injectable iron-dextran compounds for prevention and treatment of baby pig anaemia.

Results with these injectable compounds, reported by several workers, generally indicate that iron-dextran compounds are more effective for

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prevention and treatment of anaemia than iron given orally. There are, however, variations in the conclusions of investigators concerning the time of injection, the number of injections required to maintain adequate haemoglobin levels and the effects on growth.

Approximately 7 to 10 mgm. of iron are required daily by the suckling pig of which probably in the order of 1 mgm. is supplied in the sow's milk (1,3,17). One injection of iron-dextran supplying 100 mgm. of iron may be sufficient to support adequate haemoglobin levels (1,9,10) but some workers have reported that oral iron will promote development equal (6,8,18) or superior (11) to that obtained from a single injection of iron-dextran. Age at weaning may influence these results (21). Iron-dextran is usually administered when the pigs are 3 to 4 days of age, although some workers favour injection when the pigs are 7 days old (1,2).

As few workers have reported fully on the effects of various iron supplements on blood constituents, the experiment reported here was designed to compare three injectable iron compounds, oral iron in the form of paste, and sods sprinkled with an iron sulphate solution as sources of supplemental iron for baby pigs. Haemoglobin levels, haematocrit values and erythrocyte counts were used as indices.

MATERIALS AND METHODS

A total of 60 pigs representing 12 litters were involved in this experiment.

Pigs in each litter were nominated at random to each treatment, except in the case of sods. For this treatment, several litters were fed sods and from these litters a number of pigs were taken at random at 3 days of age and used throughout the experiment for blood analysis.

The following sources of iron were compared:

1. One sod (S), measuring approximately 10″ x 10″ x 4″, and sprinkled with a pint of an iron sulphate solution obtained by dissolving 15 gm. of iron sulphate in a gallon of water, was given to each litter at 3 days of age and thereafter twice a week until the pigs were creep feeding.

2. Paste Iron (P)—a dosage equivalent to 250 mgm. of ferrous sulphate was administered orally on the third day and approximately 500 mgm. on the 10th, 17th and 24th day. The paste also contained several components of the Vitamin B—complex as well as cobalt.

3. Injectable iron-dextran compound (A) containing 50 mgm. of iron as ferric hydroxide per cc. A single injection of 2 cc., supplying 100 mgm. of iron, was administered.

4. Injectable iron-dextran compound (B), similar to compound A, containing 50 mgm. of iron as ferric hydroxide per cc. A single injection of 2 cc. was administered.

5. Injectable iron in the form of ferric ammonium citrate (C). This iron source also contained several components of the vitamin B-complex. One cc., supplying thirty mgm. of ferric ammonium citrate, was administered. From the manufacturer's description on the label, it was calculated that, per recommended dose of 1 cc., this solution contained 2-3 mgm. of elementary iron. Actual analysis revealed this to be 5 mgm. per cc.* The injectable compounds A, B and C were administered in accordance with the directions of the manufacturers when the pigs were 3 days of age.

Blood samples were obtained from the anterior vena cava at weekly intervals from 3 to 45 days of age.

P—Pig Iron—Ayerst, McKenna and Harrison Ltd., Montreal, Que.
A—Imferon—Benger Laboratories Ltd., England
B—Ferrextran—Fort Dodge Lab., Inc., Fort Dodge, Iowa
C—Vita Iron—McClelland Veterinary Supplies, Calgary, Alta.

* Provincial Analyst, Edmonton, Alta., Lab. No. 59-4734.

The following blood analyses were made:

(a) Haemoglobin determination, using a Fisher electrophotometer.

(b) Erythrocyte count, using a Levy-Hausser Counting chamber with improved Neubauer double ruling.

(c) Haematocrit determination, using Wintrobe hematocrit tubes centrifuged at 3,000 r.p.m. for 30 minutes.

All pigs had access to creep-feed at 3 weeks of age and were weighed at birth, 21, 42 and 56 days of age, at which time the pigs were weaned. The data were subjected to an analysis of variance.

During the course of this experiment, the company distributing injectable iron compound A marketed a newer compound, called "Imposil" or "Imferon 75", containing 75 mgm. of iron per cc. A preliminary test of this product was obtained by injecting each pig of eight litters with 2 cc. of this solution at 3 days of age, supplying 150 mgm. instead of 100 mgm. of iron as in the case of compound A. Two pigs of each litter, a total of sixteen pigs, were taken at random and their haemoglobin levels and haematocrit values were determined once at 45 days of age.

RESULTS AND DISCUSSION

Haemoglobin Levels

Average haemoglobin levels are shown in Figure 1.

Pigs receiving sods showed a reduction in haemoglobin at 10 days of age. This probably reflected insufficient sod consumption during the first week. After 10 days, the haemoglobin levels of the pigs on this treatment increased reaching a peak at 38 days of age. Both of the iron-dextran

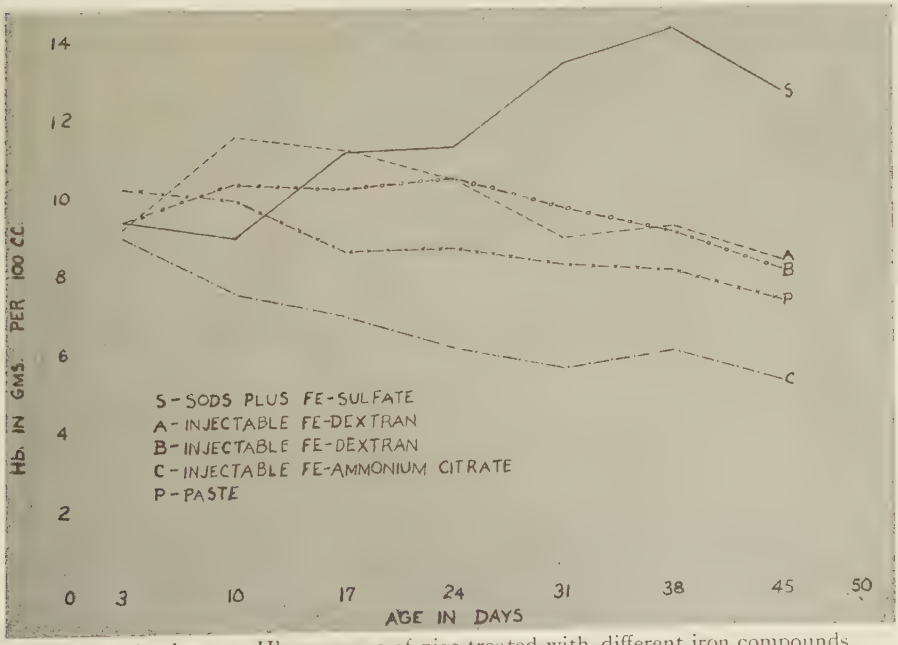

Figure. 1 Average Hb. response of pigs treated with different iron compounds.

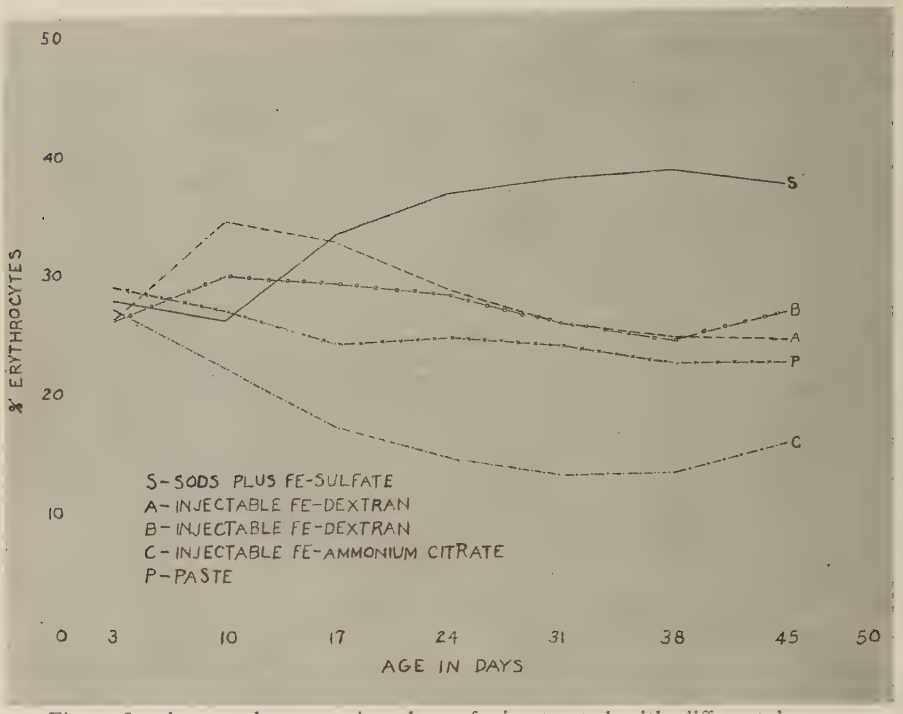

Figure 2. Average haemotocrit values of pigs treated with different iron compounds.

compounds, A and B, provided immediate increases in haemoglobin level, with the highest levels reached at 10 and 24 days of age, respectively. Thereafter there was a gradual decline to 45 days. The haemoglobin content of the pigs on the paste treatment showed a slow and almost continuous decrease from 3 to 45 days of age. Haemoglobin levels of the pigs on ferric ammonium citrate, compound C, dropped rapidly and almost continuously from 3 days of age onwards. At 45 days of age, all haemoglobin levels were below the normal values reported in the literature, except for those pigs on sods plus iron sulphate.

Haematocrit Values

The average haematocrit values for the different treatments are illustrated in Figure 2.

The pattern of the haematocrit values was very similar to that of the haemoglobin values. There was an initial drop in the case of pigs on the sod treatment and an initial rise in the case of pigs on injectable iron-dextran compound A and B. After 10 days of age, this picture was reversed: pigs on treatment S showed an increase, while those on compound A and B showed a decrease in haemotocrit values.

The haematocrit values for the pigs treated with oral iron, in the form of paste (P), decreased gradually and almost continuously from 3 to 45 days of age. Pigs on treatment C, ferric ammonium citrate, showed a

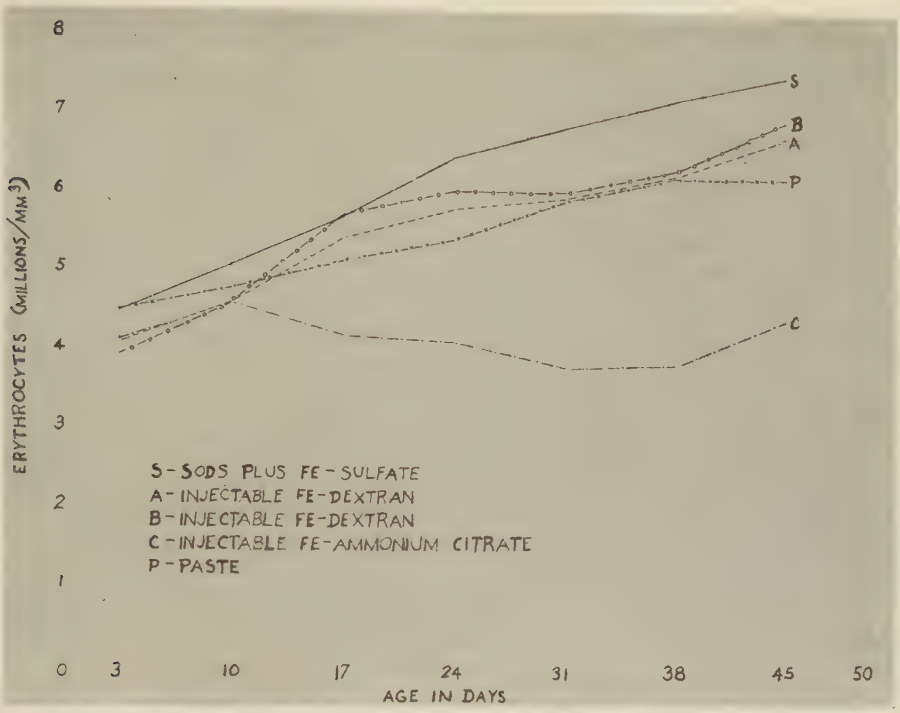

Figure 3. Average number of erythrocytes/MM³ of blood.

rapid decrease in haematocrit values from 3 to 31 days with an increase during the 38- to 45-day period. All haematocrit values, at 45 days, were below those reported as normal, except for those pigs on treatment S.

The difference between sods and injectable iron-dextran in terms of initial response indicates that it is important to have iron available at an early age if a drop in haemoglobin and haematocrit values is to be prevented. Other workers (8,14) suggest that an early injection of iron (3 to 4 days of age) is important as it is easier to maintain a certain haemoglobin level than to raise it from a lower point. Anders (1), however, claimed that an injection of iron-dextran at any early age may be toxic and suggested that an injection either at 7 or 21 days of age resulted in efficient growth. No adverse effects were observed from an early injection in the present experiment.

Figures 1 and 2 also illustrate that one injection of 2 cc. of iron-dextran compounds A and B, supplying 100 mgm. of iron, failed to maintain normal haemoglobin and haematocrit values up to 45 days of age. This is in agreement with results of other investigators, (6, 8, 11, 14). Miller (14), states that a second injection may be required during the middle or latter half of the nursing period in cases where abundant milk supply fosters rapid growth and at the same time discourages consumption of supplemental feed. Dale (6) found a second injection at 28 days of age to give a definite response with pigs weaned at that time.

TABLE 1.—THE AVERAGE MEAN CORPUSCULAR VOLUME AND HAEMOGLOBIN CONTENT OF THE INDIVIDUAL ERYTHROCYTE

Treatments	3 Days	10 Days	17 Days	24 Days	31 Days	38 Days	45 Days
Mean corpuscular volume (MCV), in cubic microns							
Sods + Fe-Sulphate	62·7	51·6	59·2	57·2	55·8	53·9	50·0
Fe-dextran A	64·8	75·6	60·9	50·1	43·7	39·9	36·4
Fe-dextran B	66·8	65·6	51·2	46·9	43·1	38·8	38·6
Fe-Amm.-citrate	66·7	48·8	42·1	35·9	35·0	35·1	35·6
Paste	64·7	56·6	47·2	45·9	40·7	36·3	36·4
Mean corpuscular haemoglobin (MCH) in micro micro grams							
Sods + Fe-Sulphate	21·1	17·6	19·7	17·5	19·6	19·9	16·9
Fe-dextran A	22·8	25·2	20·8	18·1	14·9	14·6	12·3
Fe-dextran B	23·9	22·5	17·8	17·3	16·1	14·3	11·5
Fe-Amm.-citrate	22·0	16·6	16·6	14·8	14·6	15·6	11·6
Paste	22·8	20·9	16·8	16·0	13·8	12·9	11·6

Erythrocytes

The average number of erythrocytes is shown in Figure 3.

Only the pigs treated with ferric ammonium citrate (C) showed an abnormal low number of erythrocytes. Coffin (5) points out that, in case of anaemia caused by iron deficiency (hyprochromic mictrocytic anaemia) the red cell count of anaemic animals may nearly equal that of normal animals. In anaemic animals, however, the cell size and haemoglobin content of the individual cells are lower than normal. This is illustrated in Table 1 showing the calculated mean corpuscular volume (MCV) and mean corpuscular haemoglobin content (MCH) of the individual erythrocyte.

The results in Table 1 show that at 45 days these values were all below the lower normal values reported in the literature, indicating the existence of anaemia in all treatments, except for those pigs on treatment S.

The analysis of variance showed a consistent significant within sex-treatment difference from 24 days to 45 days of age for haemoglobin, haematocrit and erythrocytes.

Growth

The effect on growth for the different treatments was not apparent for the first 3 weeks (Figure 4). At 42 and 56 days of age there was a significant difference in weight for the different treatments.

Two pigs treated with the injectable ammonium citrate solution died at 42 and 60 days of age, both exhibiting severe anaemia. Their haemoglobin levels reduced to 4.55 and 4.73 with corresponding haematocrit values of 7.4 and 9.0 and erythrocyte counts of 1.87 and 3.02 respectively. All other pigs on this treatment had an anaemic appearance and were

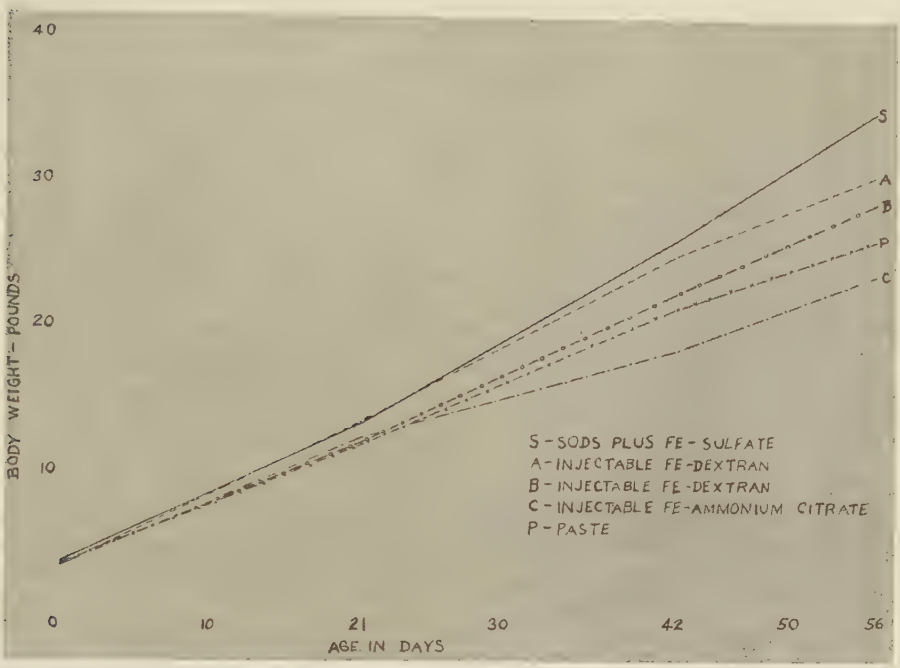

Figure 4. Average body weight from birth to weaning (8 weeks).

unthrifty. They slowly recovered after creep feed consumption increased. The poor performance of these pigs was attributed to the low iron content of the compound. It is also possible that iron in this form is not efficiently used as it has been reported that some forms of iron may not be absorbed into the body from the site of injection (2, 4, 16).

The pigs on the other treatments appeared to be in good condition with no outward signs of anaemia.

There was no visible damage evident at the site of injection with any of the three injectable compounds used. There is the possibility that under unsanitary conditions infection may be introduced at the time of injection. This could cause permanent damage to the ham, one of the most valuable parts of the carcass.

The preliminary test with Imposil indicated that this compound, when administered according to the manufacturer's directions, provided normal haemoglobin levels and haematocrit values at 45 days of age. The average haemoglobin and haematocrit values for the 16 pigs on this compound were 10.51 and 35.6 respectively, as compared with the values of 8.15 and 24.2 for the contemporary pigs receiving Imferon.

From the over-all results of this study it may be concluded that sods sprinkled with iron sulphate and fed twice a week maintained normal blood values. A single injection with iron-dextran compounds at 3 days of age, supplying 100 mgm. of iron, was not sufficient to maintain adequate blood values up to 45 days of age, although no outward signs of anaemia could be observed.

Results with oral iron in the form of a paste were slightly below those obtained with the iron-dextran compounds.

Injectable ferric ammonium citrate was highly inadequate at the levels used as an iron supplement for baby pigs. Pigs on this treatment became anaemic and unthrifty, and two, both exhibiting severe anaemia, died before the close of the experiment.

Growth rates of pigs on all treatments were similar to 21 days of age. At 42 and 56 days, the sod treatment showed a slight advantage over the iron-dextran and paste treatments and a distinct advantage over the pigs treated with ferric ammonium citrate. In the latter group growth rates were definitely retarded.

Preliminary trials with an iron-dextran compound containing 150 mgm. of iron per injection, instead of 100 mgm., resulted in normal blood values at 45 days of age.

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THE EFFECT OF ESTRADIOL AND TESTOSTERONE INJECTIONS AND THYROIDECTOMY ON MUSCLE FIBRE DIAMETERS AND LIVE WEIGHT GAIN IN YEARLING SHEEP[1]

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ABSTRACT

In this study effects of estradiol and testosterone injections and thyroidectomy on muscle fibre diameters of ewe and wether yearlings were determined. Forty-eight sheep, half of which were ewes and half wethers, were subdivided into estradiol, testosterone, and control groups containing equal numbers of each sex. Half of the animals within each sex group and treatment group were thyroidectomized. Biweekly rates of intramuscular injection of estradiol and testosterone were 5 mg. and 175 mg., respectively. All animals received the same amount of feed per day throughout a 195-day experimental period.

Live weight gain, carcass weight, and percentage dry matter of *m. longissimus dorsi* tissue were not influenced significantly by hormone injection, thyroidectomy, or sex. Estradiol injections significantly increased ($p < .01$) muscle fibre diameters of thyroidectomized ewes and wethers but the animals with thyroids were unaffected. Testosterone injections failed to affect muscle fibre diameters. Differences in muscle fibre diameters could not be explained by differences in percentage moisture content of muscle tissue.

INTRODUCTION

Meat tenderness and quality have long been associated with the size of muscle fibre bundles and the individual fibres contained therein. Following an early investigation involving small numbers (10), Hammond (8,9), and others (19, 20, 21) found a negative correlation between fibre size or meat texture and degree of tenderness. Results of previous studies also indicated that factors influencing muscle fibre size include species (10, 12, 13, 14, 16), breed (7, 10, 13), body weight (12, 13, 24), physiological and chronological age (10, 16), plane of nutrition (13, 14), individual muscles (12, 13), and sex (10, 13).

The use of hormones and antibiotics in rations of fattening livestock has resulted in carcasses differing in physical and chemical composition from those of conventionally-fed animals (6, 25, 26). The antibiotic oxytetracycline (Terramycin), when fed to wether lambs, significantly restricted muscle fibre diameter increases when fed singly or in combination with diethylstilbestrol, but the cross-sectional area of *m. longissimus dorsi* was not significantly smaller.[3] Although orally-administered diethylstilbestrol resulted in a significant increase in live weight gain and a heavier slaughter weight, muscle fibre diameters were not significantly larger than those of the control group.[3]

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This study measured the effect of the hormones estradiol, testosterone, and thyroxine (through thyroidectomy) on live weight gain, carcass weight, moisture content of muscle, and muscle fibre diameters of yearling ewes and wethers. The effect of these treatments on wool growth will be reported in another paper.

MATERIALS AND METHODS

Forty-eight, grade, Corriedale, yearling sheep, half of which were ewes and half wethers, were used in this study. They were divided into three groups of 16, on the basis of fleece grade and body weight. Ewes and wethers were distributed equally in each group and a further division was made by performing a thyroidectomy on half of the ewes and wethers in each of the three groups.

The groups were treated experimentally by injecting intramuscularly, at fortnightly intervals, the following solutions:

Lot 1—5 mg. of estradiol cyclopentylpropionate, repositol
Lot 2—175 mg. of testosterone, repositol
Lot 3—5 cc. of physiological saline.

Feeding of the sheep began on May 24, injections commenced on June 21, and the animals were slaughtered after final weighing on December 4, 1957. Each animal was individually fed a daily ration of 1.0 pound of whole oats and 1.5 pounds of good quality, chopped alfalfa hay. The calculated intakes of digestible crude protein (DCP) and total digestible nutrients (TDN) were 0.25 and 1.5 pounds, respectively. The feeding period lasted for 195 days.

One thyroidectomized ewe in the estradiol treatment group contracted a bacterial infection and died during the experiment. For statistical analyses missing values were calculated and included using the missing plot technique (23).

Immediately after slaughter, a core weighing approximately 5 grams was taken from *m. longissimus dorsi* approximately adjacent to the union of thoracic and lumbar vertebrae. The moisture content of one portion of this tissue was determined immediately by the conventional method (3). The other portion of the tissue was fixed in a vial of 10 per cent formalin and stored for 1 month before fibre measurements were made. Hammond and Appleton's technique for muscle fibre determination (8) was adopted for this study. One hundred fibres were measured, using the equipment and method described by Slen and Banky for wool fibre diameters (22).

Covariance analyses incorporating a split-plot design were employed to analyse the data.

RESULTS AND DISCUSSION

Percentage distribution of muscle fibre diameters according to hormone treatment, thyroid treatment, and sex followed the normal pattern expected for biological data and justified the use of the conventional methods for statistical analyses employed.

TABLE 1.—EFFECT OF HORMONE INJECTION AND THYROIDECTOMY ON AVERAGE LIVE WEIGHT GAIN ON YEARLING EWES AND WETHERS

Group	Ewes		Wethers	
	Thyroid-ectomized	With thyroids	Thyroid-ectomized	With thyroids
	Pounds	Pounds	Pounds	Pounds
Initial weight				
Estradiol	63.0	64.5	68.5	67.8
Testosterone	67.3	63.0	63.0	66.3
Control	62.8	62.5	70.0	67.8
Total liveweight gain				
Estradiol	47.8	42.8	43.5	42.5
Testosterone	37.0	39.5	43.0	40.0
Control	44.0	40.0	37.3	40.0
Carcass weight				
Estradiol	53	54	54	53
Testosterone	58	54	54	53
Control	56	53	55	57
Total TDN Intake				
Estradiol	288	288	288	288
Testosterone	276	288	276	288
Control	284	288	276	288

Effect of Hormone Injection and Thyroidectomy on Live Weight

Because of limited numbers from which to choose experimental animals, the average initial live weight of ewes and wethers differed significantly ($p < .01$) in the estradiol and control groups (wethers being heavier). The initial live weight difference between sexes in the testosterone treatment group was not significant (Table 1). In spite of the initial difference in live weight, sex did not have a significant effect on average live weight gain during the 195-day experimental period. However, there was a hormone treatment effect on live weight gains. Animals treated with estradiol gained more ($p < .01$) than those treated with testosterone or the control animals. Testosterone-treated animals gained less weight than control animals but the difference was not significant.

The small differences between subgroups in average total digestible nutrient intake were not statistically significant. It must be concluded that differences in live weight gain were due directly to hormone treatment. Differences in average carcass weights between treatments and sex groups were not significant in spite of an initial live weight difference between sexes.

Effect of Hormone Injection and Thyroidectomy on Percentage Dry Matter of Muscle Tissue

Tissue samples from *m. longissimus dorsi* were dried and the percentage dry matter was calculated by difference. There was no significant difference in percentage dry matter of *m. longissimus dorsi* due to hormone injection, thyroidectomy, or sex (Table 2).

TABLE 2.—EFFECT OF HORMONE INJECTIONS AND THYROIDECTOMY ON AVERAGE FIBRE DIAMETER (MICRONS) AND DRY MATTER PERCENTAGE OF MUSCLE TISSUE

Group	ESTRADIOL Muscle		TESTOSTERONE Muscle		CONTROL Muscle	
	Fibre diameter averages	Dry matter %	Fibre diameter averages	Dry matter %	Fibre diameter averages	Dry matter %
All animals	55.2[1]	26.1	51.7	25.2	49.4	26.0
Animals with thyroids	51.8	25.6	52.	25.0	51.0	26.2
Thyroidectomized animals	59.1[3]	26.6	50.7	25.5	47.7	25.8
All ewes	55.0	26.0	52.7	25.3	48.8	26.6
Ewes with thyroids	53.3	26.1	55.5	25.7	50.5	26.8
Thyroidectomized ewes	57.2[2]	25.8	49.9	24.9	47.1	26.5
Wethers	55.5[2]	26.2	50.6	25.2	49.9	25.3
Wethers with thyroids	50.4	25.2	49.8	26.1	51.5	25.6
Thyroidectomized wethers	60.5[4]	27.2	51.4	24.2	48.3	25.0

[1] Larger ($p < .05$) than testosterone and larger ($p < .01$) than control values
[2] Larger ($p < .01$) than testosterone or control values
[3] Larger ($p < .01$) than animals with thyroids that received estradiol injections
[4] Larger ($p < .05$) than wethers with thyroids that received estradiol

Few studies have presented the effect of hormones on carcass composition. Whiting, Clark and Allen (25) found a higher moisture percentage in round and rib sections of diethylstilbestrol-implanted lambs. This may have indicated a higher percentage of protein tissue with its concomitant higher moisture percentage and/or a lower percentage fat in the adipose tissue. Using lambs, Gee and Preston (6) found implanted hexosterol increased percentage bone, water, and protein content but decreased percentage fat. The increase in protein could not fully account for the increase in moisture since the moisture: protein ratio was higher in treated animals than in untreated animals.

Effect of Hormone Treatment on Muscle Fibre Diameters

In this study average muscle fibre diameters were greater than those published for sheep by Joubert (12, 13) or the values expected using his formulation (13). However, the differences were small and probably were caused by breed or plane of nutrition.

Sex, thyroidectomy, and testosterone injections all failed to have a significant effect on average muscle fibre diameters.

Responses of muscle fibre diameters were greater to estradiol injections than to testosterone injections. Average muscle fibre diameters of all subgroups exceeded those of their counterparts in the control group with the exception of wethers with thyroids. Analysis of covariance (to adjust for differences in initial body weights) indicated that the increases in fibre diameters of thyroidectomized ewes and wethers were significant ($p < .01$ and $< .05$). No response was obtained in the animals with thyroids nor were there any significant interactions.

The response of thyroidectomized ewes to estradiol resulted in greater ($p < .01$) average muscle fibre diameters than those in comparable sub-

groups in the testosterone and control groups (Table 2). There was no significant difference between hormone injection treatments upon pooling values for thyroidectomized ewes and those with thyroids intact.

Wethers that received estradiol produced muscle fibres having diameters that were larger ($p < .01$) than those of wethers either in the control or in the testosterone treatment group. The significance of the estradiol group values is due entirely to the thyroidectomized wethers.

A significant ($p < .01$) interaction between hormone injection and thyroidectomy was found upon analysing muscle fibre diameter data from wethers. However, there was no interaction between treatments in any other groups and subgroups.

Effect of Thyroidectomy on Muscle Fibre Diameters

In the estradiol treatment group both the thyroidectomized ewes and wethers had larger ($p < .01$ and $< .05$) muscle fibres than their counterparts with thyroids intact. This was not the case in the control and testosterone groups. In the case of the wethers there was a significant ($p < .01$) hormone and thyroidectomy interaction.

Effect of Sex on Muscle Fibre Diameters

Data pooled by ignoring thyroid status and hormone treatment showed no significant sex difference in average muscle fibre diameter.

Correlations of Final Body Weight, Live Weight Gain, and Carcass Weight with Muscle Fibre Diameters

Correlations of average muscle fibre diameters with final body weight, carcass weight, and live weight gain during the experimental period, either within treatment groups or as pooled data, were not significant.

In contrast to the results reported here, Joubert (12) obtained significant, high correlations between live weight and muscle fibre diameters of sheep on both a super-maintenance diet ($r = 0.909$; $p < .05$) and a submaintenance diet ($r = 0.962$; $p < .01$). He concluded that muscle fibre development is essentially a function of age-weight relationship and, within a species, carcasses of the same weight should have muscle fibres of similar size.

It is evident from the data presented that significant differences in average muscle fibre diameters were caused by an interrelationship between estradiol injection and thyroidectomy rather than by significant differences in live weight, carcass weight, or moisture content of muscle tissue. After thyroidectomy, estradiol caused a significant increase in muscle fibre diameter; in animals with thyroids, estradiol had no effect.

In this experiment no deleterious side effects were evident in spite of the high levels of estradiol injection, and it is noteworthy that failure of ewes and wethers to respond significantly to testosterone treatment is not unique to this experiment (1, 15, 17, 18) or to lambs (2, 5). It must be concluded that the response to estrogens and testosterone is complicated by dose rate, form, diet, and/or physiological status of the feeder animals.

De Bastiani and Zoppi (4) found, using rabbits, that the thyrotrophic activity of the anterior pituitary gland was enhanced by treatment with diethylstilbestrol. The prolonged administration of estrogen resulted in a premature cessation of skeletal growth, particularly in the long bones (11). Several workers (13, 14, 16) have indicated that the number of muscle fibres remains constant at all stages following birth. Considering these reports, it is logical to hypothesize that, in animals with thyroids, estradiol stimulated thyrotrophic activity sufficiently to counteract direct estradiol effects on muscle fibre diameters, while in thyroidectomized animals muscle fibre diameters increased directly as a result of estradiol injection. It is also possible that increases in muscle fibre diameter resulted directly from an estradiol-induced relative reduction in skeletal development.

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AN APPRAISAL OF ABBREVIATED METHODS OF DETERMINING THE TOTAL SOLIDS CONTENT OF MILK

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ABSTRACT

A modified Mojonnier method, and two lactometer methods, each designed to give rapid approximations of total solids content of milk from individual cows, were compared with the standard Mojonnier method. Each of the rapid methods underestimated a high solids content and overestimated a low solids content. The Cenco Rapid Moisture Tester proved to be accurate but no more rapid than the standard Mojonnier method.

INTRODUCTION

Two types of total solids tests for fresh whole milk are in current use. Gravimetric tests, in which the water is removed under controlled conditions, are capable of considerable accuracy but are generally slow and require skilled analysts. Specific gravity tests are quick and inexpensive, but not very accurate.

The usefulness of a total solids test will depend to some extent on the purpose for which it is to be used. Accuracy, speed and economy are of interest to all, although geneticists could utilize a test that ranked the samples accurately without giving absolute values. In the present studies the primary interest was in a test suitable for breeders although it was hoped the findings might have a broader application.

Numerous attempts have been made to develop new methods or to modify existing methods of measuring total solids. Leggatt *et al.* (8) have developed an oxidimetric method with ceric sulphate. Davey and Patton (3) compared the Mojonnier test with several other tests. They found some of the tests to hold promise but none to be completely satisfactory. Loewenstein (9) reported favourably on the Cenco Rapid Moisture Tester. Ashworth (1) used a method based on a titration to pH 4.0.

There is considerable interest in lactometer methods (2, 4, 5, 7, 12). Madden and Brunner (10) tested the accuracy of a small Quevenne lactometer and the Watson lactometer. The latter was found the more accurate but both lactometers showed a bias for milk with low and high values of fat content. Standard errors of estimate for the Watson lactometer were reported to be 0.14 to 0.39.

Jennings and Tarassuk (6) developed a modified Mojonnier test, using a single oven and a semi-automatic balance.

For many years the Quevenne lactometer read at 60°F. and the Babcock formula have been used in Ottawa for estimating total solids. This method was replaced in 1955 with a Quevenne lactometer reading at 86°F. using Herrington's modification (5) of the Sharp and Hart equation.

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The present project was undertaken to compare these two lactometer methods and a rapid modified Mojonnier method with the standard Mojonnier method (11) for total solids tests on samples of milk from individual cows. A few data were collected on a comparison of the Cenco Rapid Moisture Tester with the Mojonnier method.

MATERIALS AND METHODS

All cows in this test freshened during August, September and October, 1957, and were stabled throughout the entire test period. Each week during the test period samples of milk consisting of a pint of evening milk mixed with a pint of morning milk from each of 8 to 12 individual cows were delivered to the laboratory. The samples were thoroughly mixed and small portions were set aside for the Mojonnier and modified Mojonnier tests. The remainder was utilized for lactometer and Babcock fat tests. All tests were conducted on the day the samples were received.

The results of the Mojonnier total solids tests conducted in duplicate were used as the standard for all samples. Babcock fat tests were run in duplicate on all samples. In order to provide a valid estimate of measurement error, all duplicate tests were conducted independently of the original tests.

A rack with 12 large tubes was utilized for both 60°F. and 86°F. lactometer tests. Quart bottles of milk were tempered to 60°F., mixed and poured into the test-tubes in the rack. The rack was suspended in a large water bath at 60°F. The readings were made with a Rascher and Betzold Quevenne lactometer TP 60/60°F. with a built-in Fahrenheit thermometer. The milk was returned to the bottles and warmed to 86°F., remixed and poured into tubes in a bath tempered at 86°F. An Exax Quevenne Lactometer 60°/60°F. without thermometer was used for this method. Following the first readings at 86°F. the milk was returned to the bottle, remixed, returned to the tubes and read again. Any foam on the surface of the milk in the test-tube was removed by making the level of milk high enough to cause it to overflow when the lactometer was inserted. Caution was exercised when reading the lactometer to ensure its floating freely in the milk.

The formulae for converting the lactometer and Babcock fat tests to total solids are as follows:—

Babcock equation:

$$TS = 0.25\ L_{60} + 1.2F. \qquad \text{at } 60°F.$$

Herrington's Modified Sharp and Hart equation: at 86°F.

$$TS = .7782 + .2594\ L_{86} + 1.2648\ F.$$

The modified Mojonnier tester was a regular Mojonnier oven with the hot plate replaced by a heat exchange plate. Steam at 15 p.s.i. and cold water were connected to the heat exchange plate through a three-way valve. A thermocouple was soldered to the centre of the plate to measure the heating and cooling rate. A silica gel drying tube was inserted in the air intake line. A Mojonnier hot plate, porcelain solids top and

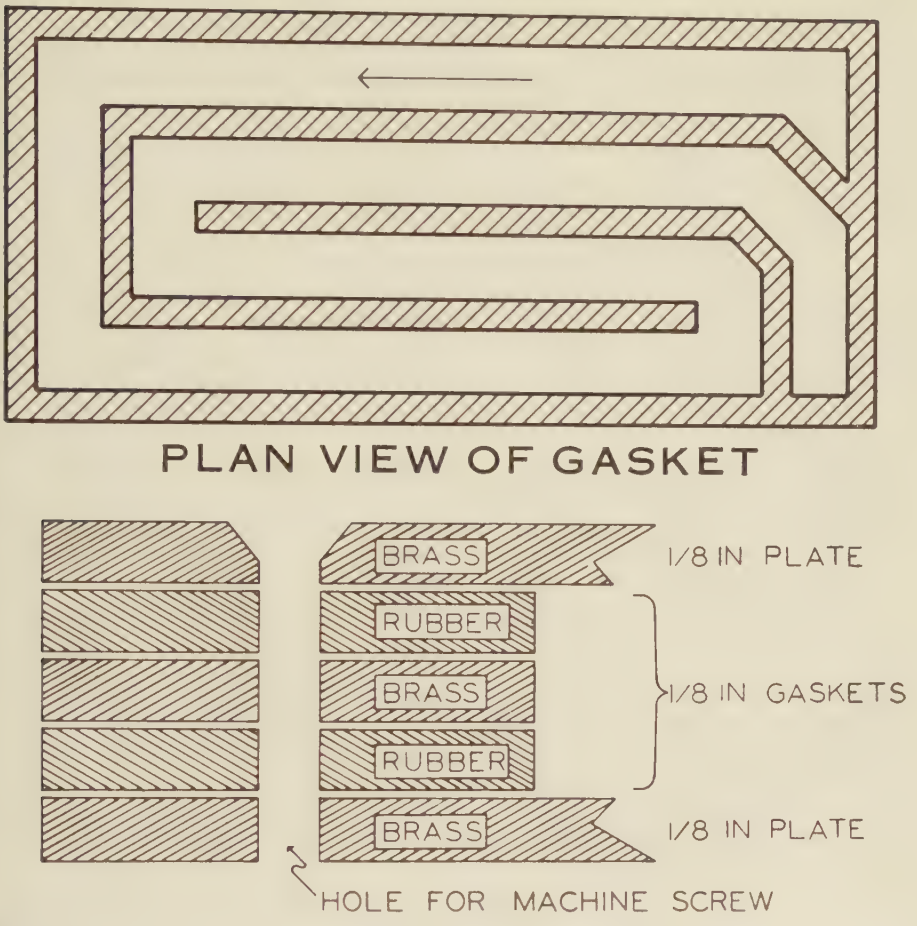

FIGURE 1. Design of heat exchange plate.

rheostat were used for the initial drying as in the regular Mojonnier procedure. All valves and switches were placed to be easily accessible to the operator. An electrical timer, 1 second to 60 minutes, and a semi-automatic balance were used in place of the standard Mojonnier items. Otherwise standard Mojonnier equipment was used. The equipment was arranged on a table constructed for this purpose.

Figure 1 shows the design of the heat exchange plate improved after Jennings and Tarassuk (6).

The modified Mojonnier test differs from the Mojonnier test in that a higher vacuum and higher temperature (105°C.) and consequently a shorter time of oven drying are used. The heat exchange plate is used for both heating and cooling.

The samples were heated under 28 inches of vacuum for 2 minutes. It required about 30 seconds to reach 28 inches of vacuum. The cooling

TABLE 1.—CORRELATIONS (r) BETWEEN DUPLICATE DETERMINATIONS ON SINGLE MILK SAMPLES

Source of variance	d.f.	Expectation of S.S.	$r=\frac{\sigma_s^2}{\sigma_s^2+\sigma_e^2}$
Mojonnier			
Among samples, within periods	194	$388\sigma_s^2 + 194\sigma_e^2$	
Within samples	212	$212\sigma_e^2$	
Variance components	$\sigma_s^2=.608$,	$\sigma_e^2=.003$	.995
Abbreviated Mojonnier			
Among samples, within periods	193	$386\sigma_s^2 + 193\sigma_e^2$	
Within samples	211	$211\sigma_e^2$	
Variance components	$\sigma_s^2=.578$,	$\sigma_e^2=.006$	.990
Lactometer 86°F.			
Among samples, within periods	110	$220\sigma_s^2 + 110\sigma_e^2$	
Within samples	120	$120\sigma_e^2$	
Variance components	$\sigma_s^2=.446$,	$\sigma_e^2=.007$	.984

water flowed through the heat exchange plate for 15 seconds which was just sufficient to cool the heat exchange plate to room temperature. The samples were left in the oven for 30 seconds to equilibrate with the plate. With the abbreviated procedure 24 determinations per hour were conducted. A Cenco Rapid Moisture Tester, operated according to the manufacturer's instructions, was used to test a few samples.

ANALYSIS AND RESULTS

Duplicate determinations on single samples of milk were conducted on 212 samples by the official Mojonnier method, 211 by the modified Mojonnier method, and 120 by the 86°F. lactometer method. Eighteen weekly periods were represented by the Mojonnier and abbreviated Mojonnier determinations and ten periods by the lactometer method. Therefore, in estimating the "among sample" variance the "among sample, within period" sums of squares were used. These variances, together with the "within sample" variance, were used to estimate the correlation among duplicates. The results of the analysis are presented in Table 1.

The first analysis presents an encouraging result indicating extreme accuracy of all methods in terms of yielding almost identical results from separate determinations on the same milk. Of course, this is a within-method analysis and critical evaluation of the methods requires a comparison of the results among methods.

Since no duplicates were performed with the lactometer at 60°F. only first determinations of duplicates for other methods were included in the second analysis. This was considered necessary for analysis of variance

TABLE 2.—SUMMARY OF ACTUAL DATA BY PERIOD AND METHOD OF TOTAL SOLIDS DETERMINATION

Period	No. observations per mean	Means of sub-class values, (per cent total solids)[1]			
		Lactometer		Modified Mojonnier	Mojonnier
		60°F.	86°F.		
1	12	12.35	12.81	12.52	12.70
2	8	12.35	12.26	12.18	12.31
3	12	12.21	12.61	12.17	12.22
4	12	11.82	12.11	11.77	11.83
5	12	12.42	12.72	12.47	12.60
6	12	12.04	12.09	11.91	11.93
7	12	11.67	12.26	12.02	12.49
8	12	12.27	12.81	12.36	12.56
9	12	12.32	12.73	12.26	12.51
10	12	11.60	12.25	11.57	11.77
11	12	12.83	13.25	12.88	13.09
12	12	12.46	12.82	12.64	12.75
13	12	12.32	12.70	12.48	12.62
14	12	12.62	12.72	12.28	12.74
15[2]	12	10.87	11.14	13.61	13.86
16	12	12.07	12.21	12.21	12.35
17	12	12.27	12.70	12.42	12.64
18	12	11.89	12.46	12.21	12.33

[1] The standard error for individual observations is .746 which is an estimate of the range on either side of the means containing 68 per cent of the observations.

[2] Period 15 was eliminated from subsequent analysis since the major discrepancy between lactometer and Mojonnier methods was ascribed primarily to measurement error.

TABLE 3.—ANALYSIS OF VARIANCE FOR METHODS AND PERIODS

Source of variation	d.f.	Mean square	F.
Total	799	.6340	
Methods	3	5.4742	9.84**
Periods	16	4.7569	8.55**
Methods × Periods	48	.1437	
Residual	731	.5561	

** Indicates a highly significant contribution of variation from methods and periods.

of method comparisons since duplicates in one case and not in another would cause heterogeneity of variance. Using only single determinations enables the use of additional lactometer (86°F.) determinations that were not conducted in duplicate. A summary of the data showing method and period averages is given in Table 2.

The within-method and period variances of these single values are .5365 (lactometer 60°F.), .5160 (lactometer 86°F.), .5723 (modified Mojonnier), and .6008 (Mojonnier). Bartlett's test applied to these variances indicated a lack of heterogeneity with a Chi squared value of .523 or a probability of acceptance greater than .75. The results of an analysis to test the differences between methods are presented in Table 3.

TABLE 4.—REGRESSION ANALYSIS OF APPROXIMATE METHODS ON THE MOJONNIER METHOD

Within period variation and covariation
(x = Mojonnier)

	d.f.	Σx^2	Exy	Ey^2	$b_{yx} \pm S_{byx}$
Modified Mojonnier	183	134.022	101.297	104.168	.756 ± .389
Lactometer 86°F.	184	134.063	97.804	94.440	.730 ± .355
Lactometer 60°F.	184	134.063	94.518	90.181	.705 ± .359

The actual method averages of per cent total solids for the data in this over-all analysis are: lactometer 60°F. 12.20; lactometer 86°F. 12.56; modified Mojonnier 12.25; and Mojonnier 12.43. It is obvious from these results that the abbreviated methods are only approximate and do not in fact estimate without bias the same milk characteristics as does the Mojonnier method. The non-significant interaction of methods × periods indicates that the ranking of methods was consistent over periods.

The ultimate value of the three short-cut methods rests on the degree to which they can be used to predict the actual Mojonnier values. An estimate of the regression of the values from the abbreviated methods on the average of corresponding duplicate Mojonnier values is shown in Table 4 together with the standard errors of the three regression coefficients.

The results of this regression analysis indicate regression coefficients smaller than 1. An unbiased prediction method for the whole range of total solids levels would have a regression coefficient of 1. Values less than 1, as found here, indicate the abbreviated methods give too high values for low-total solids milk and too low values for high-total solids milk. The magnitude of the standard errors (Sb_{yx}) shows that the bias cannot be estimated very accurately.

None of the abbreviated methods studied would be expected to always yield values within 1 percentage unit of the true Mojonnier value on the basis of single determinations on milk from individual cows. Examination of these large standard errors was attempted by comparing the separate regressions by periods with the average within-period regression. This comparison is presented in Table 5.

Here it is seen that the relationship between the approximate values and the Mojonnier values is different among periods. Thus, either the methods were not applied similarly for the different periods or the different milk characteristics during each period produce different relationships.

Fresh whole milk samples from 16 cows were tested with the Cenco Moisture Tester and the results compared with Mojonnier values from the same samples. The average of the 16 values was 12.75 per cent for the Cenco Test and 12.84 for the Mojonnier method. The regression of the Cenco values on the Mojonnier values was .993± .1969.

TABLE 5.—INDIVIDUAL PERIOD REGRESSION VS. THE AVERAGE WITHIN-PERIOD REGRESSION

	d.f.	S.S.	M.S.	F
Modified Mojonnier				
Ind. regressions	17	91.726		
Av. regression	1	76.563		
Difference	16	15.163	.948	12.640**
Σy^2, Table 3	183	104.168		
Ind. regressions	17	91.726		
Residual	166	12.445	.075	
Lactometer 86°F.				
Ind. regressions	17	82.494		
Av. regression	1	71.352		
Difference	16	11.142	.696	9.666**
Σy^2, Table 3	184	94.440		
Ind. regressions	17	82.494		
Residual	167	11.946	.072	
Lactometer 60°F.				
Ind. regressions	17	78.438		
Av. regression	1	66.638		
Difference	16	11.800	.738	10.542**
Σy^2, Table 3	184	90.181		
Ind. regressions	17	78.438		
Residual	167	11.743	.070	

** Individual period regressions account for a highly significant greater covariation than does the average within-period regression.

DISCUSSION AND CONCLUSIONS

Some of the errors in total solids determination based on density or displacement measurement can be accounted for on the basis of milk composition, temperature, state of the fat and technique. Lactometer methods are affected by errors in the fat test that the gravimetric methods avoid. It is, however, difficult to explain the bias exhibited by the methods under investigation.

The close agreement between duplicates as illustrated in the first analysis indicates good technique. From this it may be judged that differences in milk characteristics among periods caused the heterogeneity among regression coefficients. Stages of lactation advanced throughout the test period but feeding and management were not altered. The fact that total solids values were found to be significantly different among periods emphasizes the possibility of the relationship between the approximate methods and the Mojonnier method being affected by changes in milk characteristics. This is substantiated by the heterogeneity of the "within period" regression coefficients.

The remarkably accurate results using the Cenco Tester speak well for the method but samples are tested one at a time and require about 10 minutes each, so the slowness of operation detracts from its practicability. Furthermore, repeated tests of the method may also indicate significant variation from period to period as was found in our more extensive comparisons with the two lactometer and modified Mojonnier tests.

The two lactometer methods and the modified Mojonnier method, as conducted in this study, lack the accuracy necessary to make absolute determinations of the total solids content of samples of milk from individual cows. Considering the extreme range of errors to be ± 1.0 per cent total solids, at least five tests per lactation are needed using the lactometer or modified Mojonnier method to rank cows on their total solids production with a bare minimum of accuracy.

It is evident from our results that comparisons of this kind must be replicated over periods to fully appraise the relative usefulness of different milk-testing procedures. The analysis of results should, in addition to comparing the over-all averages, make comparisons at different levels of the standard values. Other authors have credited the short-cut methods tested here with greater accuracy than is possible from our results. It is suspected that this may be caused by differences in comparison procedures.

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THE PERFORMANCE OF LAYING HENS REARED ON RESTRICTED AND FULL FEEDING PROGRAMS

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ABSTRACT

Experiments were conducted in 3 successive years to measure the effect on the performance of laying hens of restricting feed during the rearing period on range. About 1100 birds were reared and carried through a laying year in each experiment. Feed restriction was 72, 76 and 63 per cent of full feed for the test groups in Experiments 1, 2, and 3 respectively. All birds were full-fed in the laying house.

Feed restriction changed the pattern of egg production, in that the restricted birds came into production later but subsequently laid at a higher rate than their full-fed counterparts. After 318 and 322 days in production the restricted birds in Experiments 1 and 2 respectively had overcome the disadvantage of a late start (7 and 14 days) and produced as many eggs as the full-fed birds. In Experiment 3, the restricted birds were held back 20 days, and after 315 days in production were still significantly behind in number of eggs produced.

The restricted birds in the three experiments consumed an average of 0.17 pounds less feed per dozen eggs over the combined rearing and laying periods. This amounts to a saving of 3 pounds of feed per bird, since the mean egg production of the restricted groups was 18 dozen eggs per bird.

Early egg size was markedly increased by feed restriction and egg weights taken later in the year (after 3 months in production) remained slightly higher for the restricted groups. Albumin score for eggs from the restricted birds was better in all tests, but not significantly so in any given experiment. Specific gravity of the eggs was not influenced by feed restriction.

Mortality on range and in the laying house was not affected by feed restriction on range. Body weight at housing time was much lower for the restricted birds but was not significantly lower at the end of the laying year.

INTRODUCTION

The practice of rearing pullets on a low plane of nutrition has become increasingly popular in recent years. Restriction of nutrient intake can be achieved in a variety of ways, such as by feeding an incomplete diet, by feeding a highly fibrous diet, by quantitatively limiting the intake of a standard growing ration or by any combination of these methods.

Practically all investigators reporting on the subject agree that restricting by whatever means the nutrient intake of growing pullets results in a delay in sexual maturity and a decrease in body weight which is rapidly made up when the birds go on full feed in the laying house. There is less agreement regarding other effects of restricting the nutrient intake of growing pullets.

Milby and Sherwood (2) concluded that restricting the feed of growing pullets had no real effect on subsequent egg production, egg weight on any given date, final body weight or mortality, nor was there any appreciable saving in feed. Sunde *et al.* (4) found similar results, except that they obtained larger eggs in the early production period from the restricted

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birds. Schneider *et al.* (3) on the other hand found that restricted birds had considerably lower mortality in the laying house, over-all feed consumption was lower, and the pattern of egg production was shifted in that the restricted birds came into production later and then laid more intensively, although total egg production was not affected. Temperton and Dudley (5) reported a saving in over-all feed consumption due to restriction in the rearing period but no effect on total egg numbers.

Differences in the findings of various workers investigating this subject are probably due in part to different methods of expressing results and in part to differences in experimental conditions. In the present study, an attempt was made, by conducting experiments in 3 successive years, to determine the most consistent effects of restricted feeding in the rearing period. The method and severity of restriction varied from year to year, but this was not regarded as an undesirable feature of the design of the tests, being in keeping with the objective of testing restricted feeding under a variety of conditions to determine its main effects.

MATERIALS AND METHODS

The same facilities were used and the same general plan was followed in all experiments. Early in July over 1200 pullet chicks, 8-9 weeks of age, were divided between two adjoining 2-acre pastures. The birds on one pasture were full-fed while a restricted feeding program was imposed on the other group.

At approximately 21 weeks of age a few birds with gross abnormalities were removed from each group. From the remainder the birds were selected in a random manner for the laying pens located in two similar houses containing ten pens each. Five pens in each house held birds from the restricted group and five contained birds from the full-fed group.

In the first experiment equal numbers of purebred Single Comb White Leghorns, and a commercial strain of Hybrids, were employed. The two types of birds were equally represented in each rearing treatment group and in each laying pen. In the second and third experiments all the birds were Single Comb White Leghorns.

Table 1.—Design of experiments

Experiment No.	1		2		3	
Time on range (days)	88		92		85	
Age at housing (days)	151		156		144	
Range feeding program	Restricted	Full-fed	Restricted	Full-fed	Restricted	Full-fed
Birds placed on range (no.)	670	670	828	827	700	701
Birds housed (no.)	560	560	600	600	500	500
Birds per pen (no.)	56	56	60	60	50	50
Replicates (no.)	10	10	10	10	10	10

Details of the design of each experiment are outlined in Table 1. The specific methods and procedures employed during the rearing and laying periods are described below.

Range Rearing Procedures

In the first experiment the level of restriction was constant throughout the range rearing period, amounting to a 25 per cent reduction calculated weekly. In Experiments 2 and 3 restriction was more severe at the end of the range period than at the beginning, particularly in the case of Experiment 3. The restriction procedures used in each experiment were as follows:

Experiment 1

The restricted group in this experiment consumed 72 per cent as much feed as the full-fed group over the entire period on range. Restriction was achieved by allotting 75 per cent as much mash and 75 per cent as much grain (in the form of whole oats) as had been consumed by the full-fed group the previous week. The weekly mash allotment was fed in six equal portions each morning for 6 days and the weekly grain allotment was fed in equal portions twice a day for 6 days and once on the seventh day. The full-fed group received growing mash, wheat and oats in separate hoppers free choice throughout the period on range.

Experiment 2

The restricted birds in Experiment 2 consumed 76 per cent as much feed as the full-fed birds. They were allowed 6 pounds of growing mash and 6 pounds of oats per 100 birds per day for the first 7 weeks on range, and 7 pounds of mash and 7 pounds of oats for the last 5 weeks. The full-fed birds received equal amounts of mash and oats in such quantity as they would clean up each day.

Experiment 3

In Experiment 3, the restricted group ate 63 per cent as much feed on range as the full-fed group. They were fed free choice the first 2 weeks, and in succeeding weeks received a progressively decreasing percentage of the amount eaten by the full-fed group the preceding week, amounting to 80 per cent for weeks 3 and 4; 70 per cent for weeks 5, 6 and 7; 60 per cent for weeks 8, 9 and 10; and 50 per cent for weeks 11 and 12. The mash was fed in equal portions each morning, and the grain in equal portions each evening. The full-fed group received mash and whole oats in separate hoppers free choice.

Laying House Procedures

In each experiment the birds were confined to the laying pens for a period of 336 days. The all-mash system of feeding was used, and feed, grit and water were provided free choice. In the winter months artificial lighting was provided to give a 13-hour day. No culling was practised during the laying period.

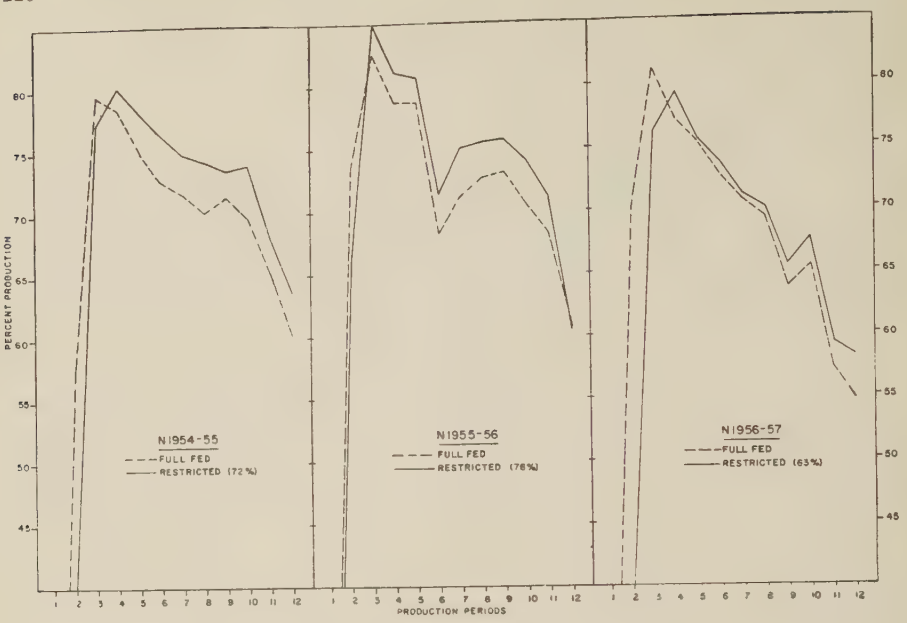

FIGURE 1. Egg production of birds reared on restricted and full feeding programs.

Five treatments were imposed during the laying period in each experiment. These treatments consisted of different levels of protein and energy in the diet. However, since birds reared on the restricted and full-feeding programs were equally represented in each laying house treatment, and since there were no interactions between range and laying house treatments, the latter are not discussed in this paper.

Laying house performance was measured in terms of egg production, feed consumption, mortality and body weight. Data were also obtained on egg weights, egg shell quality and interior egg quality.

RESULTS AND DISCUSSION

The rearing period in these experiments was considered to extend from the time the birds were placed on range until they reached sexual maturity. Time of sexual maturity in turn was taken as the age at which the restricted or full-fed groups reached a level of 2 per cent production, which in most cases did not occur until some time after the birds were in the laying house and on full feed.

Egg Production

The effects of feed restriction on egg production are illustrated graphically in Figure 1, in which per cent egg production is plotted for successive 28-day periods. Feed restriction caused a delay in the onset of egg production, but after coming into lay the restricted birds produced at a higher rate than the full-fed birds.

This change in the pattern of egg production, first reported by Schneider *et al.* (3), is the most significant effect of feed restriction on egg

TABLE 2.—EGG PRODUCTION OF BIRDS REARED ON RESTRICTED AND FULL FEEDING PROGRAMS

Expt. No.	Rearing program	Production to a given age		Production for equal periods	
		Time in prod. (days)	Eggs per bird[1] (no.)	Time in prod. (days)	Eggs per bird[1] (no.)
1	Restricted	318	217.8	318	217.8
	Full fed	325	217.7	318	213.5
2	Restricted	322	229.0	322	229.0
	Full fed	336	228.9	322	221.6
3	Restricted	315	206.4	315	206.4
	Full fed	335	218.4	315	206.7

[1]Hen-day basis

production. It explains why there might be disagreement among investigators on the effect of feed restriction on the number of eggs produced. It is evident from Figure 1 that the relative number of eggs produced by the restricted and full-fed birds depends upon the time at which the comparison is made. The restricted birds are at an early disadvantage because of a later start but they gradually overcome this disadvantage as the laying period progresses.

Given sufficient time, one would expect the restricted birds to lay as many eggs as the full-fed birds. This occurred in the first two experiments (Table 2) but not in the third, where production was significantly lower for the restricted birds. It may be that Experiment 3 was terminated before the restricted group had time to overcome the disadvantage of a 20-day delay in sexual maturity, but there are also indications that restriction was too severe in this experiment. The restricted birds never reached as high a peak as the full-fed group and, although they laid at a higher rate for most of the production period, their advantage was not as great as that of the restricted birds in the first two experiments. This is reflected in the fact that, over periods of equal length following sexual maturity, the restricted birds in Experiment 3 laid the same number of eggs as the full-fed birds, whereas on the same basis the restricted groups in the first two experiments laid more eggs (Table 2).

Feed Consumption

Over the combined rearing and laying periods, the restricted birds consumed less total feed in all experiments (Table 3). Most of the difference was accounted for by the restricted birds consuming less feed in a shorter laying period. Only in the first experiment did feed restriction on range reduce the amount of feed required to bring the birds to sexual maturity. After sexual maturity, the shorter laying period seemed to be responsible for most of the saving in feed, since there was little difference between the restricted and full-fed birds when compared over production periods of equal length.

TABLE 3.—FEED CONSUMPTION[1] OF BIRDS REARED ON RESTRICTED AND FULL FEEDING PROGRAMS

Expt. No.	Rearing program	Feed to sexual maturity lb./bird	Production to a given age		Production for equal periods	
			Laying period lb./bird	Laying and rearing lb./bird	Laying period lb./bird	Laying and rearing lb./bird
1	Restricted	14.8	92.2	107.0	92.2	107.0
	Full fed	16.7	94.3	111.0	92.4	109.1
2	Restricted	16.9	94.6	111.5	94.6	111.5
	Full fed	17.0	97.9	114.9	94.1	111.1
3	Restricted	14.8	96.4	111.2	96.4	111.2
	Full fed	14.5	104.8	119.3	98.0	112.5

[1]Pounds of feed per bird calculated on a hen-day basis

TABLE 4.—FEED EFFICIENCY OF BIRDS REARED ON RESTRICTED AND FULL FEEDING PROGRAMS

Expt. No.	Rearing program	Pounds of feed per dozen eggs			
		Production to a given age		Production for equal periods	
		Laying period	Laying and rearing	Laying period	Laying and rearing
1	Restricted	5.08	5.99	5.08	5.99
	Full fed	5.20	6.20	5.19	6.22
	Difference	0.12	0.21	0.11	0.23
2	Restricted	4.95	5.88	4.95	5.88
	Full fed	5.13	6.10	5.10	6.09
	Difference	0.18	0.22	0.15	0.21
3	Restricted	5.60	6.56	5.60	6.56
	Full fed	5.76	6.64	5.69	6.62
	Difference	0.16	0.08	0.09	0.06

Feed efficiency expressed in terms of pounds of feed per dozen eggs was also better for the restricted birds in all experiments (Table 4). In this case, the restricted birds retained their advantage whether compared on the basis of production to a given age or production for periods of equal length. Over the combined rearing and laying periods, the restricted birds in the three experiments averaged 0.17 pounds less feed per dozen eggs. Since mean egg production of the restricted groups was 18 dozen eggs per bird, this amounts to a feed saving of approximately 3 pounds per bird.

Mortality

Mortality on range was not influenced by feed restriction, and laying house mortality was appreciably different only in Experiment 2, where

TABLE 5.—MORTALITY AND MEAN BODY WEIGHTS OF BIRDS REARED ON RESTRICTED AND FULL FEEDING PROGRAMS

Expt. No.	Rearing program	Mortality			Mean body weights	
		Range	Laying house			
		%	%	Av. life (days)	Housing lb.	Final lb.
1	Restricted	2.5	17.5	302	3.2	4.8
	Full fed	4.0	17.5	307	3.5	4.9
2	Restricted	10.0	14.0	320	3.1	4.5
	Full fed	8.0	19.6	308	3.6	4.7
3	Restricted	3.7	21.4	303	2.4	4.3
	Full fed	2.6	22.6	302	3.3	4.4

TABLE 6.—EGG WEIGHT, ALBUMEN SCORE AND SHELL QUALITY OF EGGS LAID BY HENS REARED ON RESTRICTED AND FULL FEEDING PROGRAMS[1]

Expt. No.	Rearing treatment	Egg weight, gm.	Albumen score	Specific gravity
1	Restricted	56.5	2.90	1.082
	Full fed	56.2	2.96	1.081
2	Restricted	55.2	2.74	1.083
	Full fed	54.8	2.81	1.082
3	Restricted	54.0	2.87	1.083
	Full fed	53.5	2.97	1.083

[1]Approximately 1600 eggs were measured for egg weight and specific gravity, and 800 for albumen score per treatment in each experiment.

it was lower for the restricted birds (Table 5). It must be concluded that, under the conditions of these experiments, feed restriction had no significant effect on range or laying house mortality.

This conclusion regarding the effect of feed restriction on rearing mortality may not hold under exceptionally severe conditions. It has been the experience of commercial poultrymen that if birds being reared on a restricted program are exposed to stress of disease or excessively low temperatures, mortality will be increased if the birds are not placed on full feed over the stress period. No such stresses were encountered in these experiments.

The results of these experiments with respect to laying house mortality agree with those of most other workers, although Schneider *et al.* (3) reported 13.2 per cent lower mortality in the laying house for birds reared on a low plane of nutrition, and many commercial operators claim lower mortality. Since there are no reports of higher mortality following restricted feeding, it seems likely that if it has any effect it is beneficial, but this effect may appear only under certain conditions of stress.

TABLE 7.—EARLY EGG SIZE OF BIRDS REARED ON RESTRICTED AND FULL FEEDING PROGRAMS

Rearing program	Number of eggs	Per cent eggs in grade				Average weight, gm.
		Large	Medium	Pullet	Pee-Wee	
Restricted	First 500	4	36	48	12	49.3
	First 1000	4	43	43	8	50.1
	First 1500	8	48	38	6	50.8
Full fed	First 500	3	11	53	33	46.4
	First 1000	3	19	53	25	47.4
	First 1500	4	28	50	18	48.4

Body Weight

A reduction in body weight at housing time is an invariable result of restricted feeding and is followed by a very rapid increase in body weight when the birds are placed on full feed (Table 5). In these experiments the weight of the restricted birds was not significantly lower than that of the full-fed birds after 8 weeks in the laying house. However, in all experiments the restricted birds ended the experiment slightly lower in mean body weight than the full-fed birds.

Egg Quality and Egg Size

The effects of feed restriction on egg quality and egg weight are shown in Table 6. Shell quality was measured in terms of specific gravity determined on all eggs laid on one day during the months of December, February, April, June and August, and was not influenced by restricted feeding. Interior egg quality was measured by the U.S.D.A. visual method (1) on 20 eggs from each laying pen taken five times during the year in the months mentioned above. In each experiment the eggs from the restricted birds were slightly but not significantly better in albumen quality.

The mean egg weights shown in Table 6 represent the average of all eggs laid on 4 consecutive days after the birds had been in production approximately 3 months. In every experiment the restricted birds laid heavier eggs, although here again the difference was not statistically significant within each experiment.

A more important observation was the fact that feed restriction had a marked effect on early egg size. In Experiment 3 all eggs were graded for size during the first few weeks of production and the percentages falling into each grade are shown in Table 7. It will be noted that the full-fed birds laid approximately three times as many "pee-wee" eggs as did the restricted birds. This increase in early egg size would have an important effect on economic returns.

CONCLUSIONS

The main effects of restricted feeding on range as observed in these experiments were: (1) an increase in early egg size; (2) a saving in feed, and (3) a change in the pattern of egg production marked by a delay in sexual

maturity followed by a more intense production rate. There was also evidence that restricted feeding favourably influenced egg albumen quality and egg weight later in the production period, and unfavourably influenced final body weight, but these effects were so small as to be of little or no economic importance.

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THE EFFECT OF RELATIVE PROTEIN AND ENERGY CONTENT OF DAIRY RATIONS ON PRODUCTION AND COMPOSITION OF MILK[1]

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ABSTRACT

Two trials, each with 12 Holstein cows in early lactation, were conducted to study the effect on yield and composition of milk when the relative protein and energy contents of dairy rations were altered. A change-over design of experiments was used and digestibility of representative rations was determined with steers. A significant increase in fat-corrected milk production was obtained when energy (T.D.N.) intake was raised 5 to 12 per cent above recommended feeding standards. The protein content of the milk was increased ($P<0.05$) when digestible crude protein was 20 to 30 per cent in excess of recommended allowances, but increases in milk production, in percentages of butterfat and solids-not-fat were not significant ($P>0.05$). When molasses comprised up to 18 per cent of dry matter of a ration, digestion co-efficients for protein and fibre were reduced.

INTRODUCTION

Hills and associates (7) gave an extensive review of earlier reports of the effects of dietary protein levels on milk production of dairy cows. Their own observations indicated that increased protein levels in the ration resulted in small percentage increases in production. Perkins (11) reported similar trends. In each case the low protein levels supplied, in addition to maintenance requirements, approximately 1.25 times as much protein as the milk contained. Savage (15) and Harrison and Savage (6) reported that concentrate rations containing 16, 20, and 24 per cent crude protein did not significantly affect milk yields. An abrupt change from 16 to 24 per cent protein did not increase production. Cary (2) found that the level of protein in the ration influenced milk yields when fed with energy levels in excess of feeding standards. Similar results were obtained by Perkins (11) who showed that levels of protein intake below prescribed feeding standards gave good production on high levels of energy intake. Bratton *et al.* (1) showed that the quality of the protein in normal dairy rations did not have an important effect on milk production.

Under usual feeding practices milk composition is not appreciably affected by plane of nutrition. Abrupt changes in the ration may cause a marked temporary change in the fat content (4, 15, 16). Rowland (14) has shown that feeds containing either 60 per cent of normal protein or 75 per cent of normal energy, significantly lowered the solids-not-fat content of the milk. Reduced energy in the ration primarily affected the protein content of the milk.

The experiment reported here was designed to study the relative and associate effects of crude protein and energy on milk yields, milk composition and body weight change.

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MATERIALS AND METHODS

Two trials were conducted, each with 12 Holstein cows past peak production stage (40-113 days post-partum) which had completed at least one lactation.

A change-over design by Cochran *et al.* (3) was used for each trial. The procedure of Lucas (8) was used to establish feed intake and levels of production during preliminary periods of 3 weeks when all cows received uniform rations of hay, grass silage, and meal to individual requirements. Feed levels were adjusted at each change-over period according to the equalized feeding method of Lucas (8). The cows were allotted to three groups of 4 cows each. Cows within each group were matched as to age, stage of lactation, daily yield and body weight. The four experimental rations were allotted at random to the cows in each group.

Trial 1 was conducted over four experimental periods of 4 weeks each, interspaced by 1-week change-over periods. For Trial 2 the four experimental periods were of 3 weeks' duration, with 2-week change-over periods. The cows were weighed on 2 consecutive days at the beginning and close of each trial. Single weights were taken at the beginning and close of each experimental period. Twice-a-day milking was recorded. Samples of all feed ingredients were collected for proximate principle analysis at the end of the second week of each experimental period. At similar intervals, evening and morning milk samples were taken for determinations of butterfat, solids-not-fat, and protein (N x 6.38).

Digestibility trials were conducted with steers fed rations similar to those fed to the cows in Trial 1. Co-efficients of digestibility thus obtained were applied to the rations fed the cows in Trial 1 to evaluate digestible crude protein (D.C.P.) and total digestible nutrient (T.D.N.) intake. Owing to lack of facilities to determine digestibility of rations fed during Trial 2, average digestion coefficients (9) were used to evaluate intake.

TABLE 1.—DRY MATTER COMPOSITION OF FEED MIXTURES AND CORRESPONDING DIGESTIBILITIES OF PROXIMATE PRINCIPLES (TRIAL 1)

Ingredients	A Normal protein-low energy	B High protein-high energy	C Normal protein-high energy	D High protein-low energy
Oat-barley	5.0	26.7	27.2	5.4
Oil cake	7.6	5.2	0.3	15.7
Grass silage	49.9	39.0	14.7	50.7
Corn silage	—	—	19.9	—
Molasses	14.8	12.1	17.9	4.7
Hay	22.7	17.0	20.0	23.5
	100.0	100.0	100.0	100.0
	Digestion Coefficients			
Protein**	64	60	48	70
Fat	61	64	67	64
Fibre**	55	47	51	54
N.F.E.	64	67	65	65

**Highly significant differences in digestibility (P = 0.01)

The experimental ration allowances were based on preliminary chemical analysis of the feed ingredients to which average digestion coefficients (9) were applied to estimate D.C.P. and T.D.N. content. Estimated net energy (E.N.E.) values for the rations were also calculated by using values for corresponding feeds from Morrison's tables (9), corrected for dry matter content. Milk yields were corrected to a 4 per cent fat-corrected milk basis.

Trial 1

Table 1 shows that there were four rations in which normal and high protein were combined with low and high energy levels.

Roughages consisted largely of grass silage and corn silage with up to 9 pounds of hay fed per cow daily. Linseed oil meal and molasses were added to a basic ration of equal parts oats and barley to adjust the protein and energy content.

Trial 2

Due to the large quantities of molasses used to provide high energy levels and the resulting effect on digestibility in Trial 1, ground corn was used in place of molasses as an energy source in Trial 2. This adjustment to a more practical type of ration made it difficult to obtain low energy rations and maintain a balance of other factors. In Trial 2, therefore, the two energy levels were normal and high and not low and high as in Trial 1.

In other respects the procedure with milking cows in this trial was conducted as described for Trial 1.

TABLE 2.—LEVELS OF AVAILABLE PROTEIN AND ENERGY FROM RATIONS IN RELATION TO REQUIREMENTS AND PRODUCTION (Mean amounts cow/4-week period Trial 1)

Ration Treatment		A Normal protein-low energy	B High protein-high energy	C Normal protein-high energy	D High protein-low energy
Requirements N.R.C. stand. (10)					
D.C.P.	lb.	58.4	60.6	58.5	59.4
T.D.N.	lb.	543.9	575.4	556.7	542.1
E.N.E.	th.	471.8	505.5	486.7	477.9
Available					
D.C.P.	lb.	60.8	73.7	44.1	77.6
T.D.N.	lb.	491.6	607.0	532.2	465.3
E.N.E.	th.	495.8	657.9	619.5	453.4
Intake as percentage of requirements					
D.C.P.		104	122	75	131
T.D.N.		90	105	96	86
E.N.E.		105	130	127	95
Production					
F.C. milk	lb.**	845.6	955.2	892.4	866.6
Body change	lb.**	−26.4	17.0	−10.3	−16.0

**L.S.D. — F.C. milk 70.7 lb. (P = 0.01)
Body wt. 17.2 lb. (P = 0.01)

RESULTS AND DISCUSSION

Trial 1

Nutrient Levels and Digestibility

Table 1 shows the average dry matter composition of the ingredients in the rations fed to the cows, together with the corresponding digestion coefficients for protein, fat, fibre, and nitrogen-free extract, obtained with steers.

There was a marked difference in the digestibility of protein and fibre in the four rations. Digestion coefficients were appreciably lower for protein in ration C and for fibre in ration B. Foreman and Herman (5) showed that high levels of molasses decreased protein digestibility and that the digestibility of fibre was influenced by the source of roughage. The proportion of molasses in ration A fed to the cows was higher on the average than it was in the rations fed the steers. Thus the digestion coefficients obtained with steers might indicate an erroneously high digestibility for ration A.

The calculated available levels of D.C.P. and energy evaluated as pounds T.D.N. and therms E.N.E. are shown against requirement and resulting production in Table 2. D.C.P. and T.D.N. intakes were calculated by applying the respective digestion coefficients from Table 1 to the amounts of each ration consumed during the experimental periods. The levels of digestible crude protein and energy so determined differed somewhat from the intended levels. This was due in part to the necessary adjustment of the ration intake during the preliminary feeding periods, to differences in analyses of preliminary feed samples and samples taken during the experimental periods, and also to the difference between the digestion co-efficients used to formulate the rations and those obtained with the steers and applied to the intake data. However, the variation of nutrient intakes obtained should represent the range of most practical feeding regimes.

The high proportion of molasses in ration C had the apparent effect of reducing the available digestible crude protein and to a lesser extent the available T.D.N.

Ration Effects on Yield and Body Weight

Analyses of variance of milk yields and body weight change showed that ration effects on each of these criteria were highly significant. Since ration influence on body weight was significant, milk yield alone would not provide a true estimate of ration effects (3). Therefore, to evaluate ration trends in this trial, yield and weight must be considered together.

High protein and high energy (ration B) gave the highest milk yield along with an increase in body weight. High protein with low energy (ration D) did not significantly increase production over normal protein and low energy (ration A). With each of these rations there was a loss in weight, the greater loss occurring with the lower yields from ration A. The resulting low protein and energy values for ration C in relation to feeding standard requirements, which gave the second highest yields and a moderate loss in body weight, would indicate that there was a better utilization of nutri-ents from this ration than was represented by the digestion co-efficients.

TABLE 3.—INTERACTION ANALYSIS—PROTEIN AND ENERGY EFFECTS ON F.C. MILK YIELDS (Trial 1)

Source of variation	D/F	M.S.	F.
Rations	3	27,141.4	
Protein	1	21,092.5	6.4*
Energy	1	55,094.3	16.8**
Interaction	1	5,237.6	1.6
Error	18	3,276.3	

*Significant at P = 0.05
**Significant at P = 0.01

Relative Influence of Protein and Energy on Production

The factorial design of this trial provided a means of determining the effect on milk yields of protein, energy, and their possible interaction. This analysis is shown in Table 3.

The "F" values in Table 3 showed the influence of the energy complement of the ration to be significant at P = 0.01 and that of protein significant at P = 0.05. Interaction effects were not significant at P = 0.05. The fact that the protein had some effect on production at the levels fed is in agreement with the recent results of Reid and Holmes (13). These authors showed a small effect from protein with higher levels of concentrate feeding.

The mean effects of rations on yields are shown in Table 4.

Efficiency of Rations

Based on the calculated digestible crude protein available for milk production and the calculated protein in the milk, an evaluation was made of the efficiency of the respective rations in the utilization of protein for production. The D.C.P. available for milk production was determined by deducting the amount required for maintenance from the total available D.C.P. These values in order of rations for an average 4-week period were as follows:

Rations	A	B	C	D
	lb.	lb.	lb.	lb.
Protein in excess of maintenance requirements	40.3	52.9	23.5	57.1
Protein in milk	24.6	29.4	26.1	25.5
Difference	15.7	13.5	−3.6	21.6

These data provide some indication of the utilization of crude protein in relation to the respective levels of protein and energy fed. The negative difference between the protein available for milk production and the protein contained in the milk (Ration C) further indicates that the digestibility of protein obtained with steers may have been lower than the actual digestibility by the cows. Although the cows did lose weight while on ration C

TABLE 4.—MEAN EFFECTS OF RATIONS ON YIELDS (F.C. milk per cow/4-week period)

			Protein		Mean
			Normal	High	
Energy	High	lb.	(C) 892.4	(B) 955.2	923.8
	Low	lb.	(A) 845.5	(D) 866.6	856.1
	Mean	lb.	868.9	910.9	

there were greater weight losses exhibited while on rations A and D (Table 2). Taking into account that the actual utilization of protein and energy may have been somewhat higher than shown in Ration C, the higher trend in production coupled with a low weight loss indicates that the levels of protein and energy used resulted in this being the most efficient ration. The data for ration D indicate a high wastage of protein.

Trial 2

Nutrient Levels

The levels of available nutrients in relation to requirements for maintenance and milk production, calculated in terms of D.C.P., T.D.N., and E.N.E., are shown in the first part of Table 5. The ranges of protein and energy intake significantly influenced milk production ($P = 0.05$) as shown in the lower part of Table 5. The effect of rations on body weight change was not significant.

TABLE 5.—LEVELS OF FEED INTAKE IN RELATION TO REQUIREMENTS (Mean amounts cow/3-week period, Trial 2)

Ration / Treatment		A Normal protein-normal energy	B High protein-high energy	C Normal protein-high energy	D High protein-normal energy
Intake					
D.C.P.	lb.	41.9	45.7	37.6	49.1
T.D.N.	lb.	377.8	421.9	413.9	374.0
E.N.E.	th.	356.0	369.5	379.2	335.9
Requirements					
D.C.P.	lb.	38.2	40.1	40.1	39.3
T.D.N.	lb.	362.7	376.2	381.4	368.8
E.N.E.	th.	314.8	327.7	332.4	321.4
Intake as percentage of requirements					
D.C.P.	%	109.7	114.0	93.8	124.9
T.D.N.	%	104.2	112.2	108.5	101.4
E.N.E.	%	113.1	112.8	114.1	104.5
Ration effects on production					
F.C. milk	lb.*	517.0	557.6	578.2	540.8
Body gain	lb.	2.6	4.9	14.0	−5.7

*L.S.D. = 38.6 lb. F.C. milk at $P = 0.05$

TABLE 6.—INTERACTION ANALYSIS—PROTEIN AND ENERGY EFFECTS ON F.C. MILK YIELDS (Trial 2)

Source of variation	D/F	M.S.	F.
Rations	3	8,076.5	
Protein	1	31.5	N.S.
Energy	1	18,294.9	9.9**
Interaction	1	5,903.0	3.2
Error	18	1,848.7	

**Highly significant at P = 0.01

TABLE 7.—MEAN EFFECTS OF RATIONS ON YIELDS (F.C. milk per cow/3-week period)

			Protein		Mean
			Normal	High	
Energy	High	lb.	(C) 578.2	(B) 557.6	570.9
	Normal	lb.	(A) 517.0	(D) 540.8	528.9
	Mean	lb.	547.6	549.2	

Ration Effects on Yield and Body Weight

The trend of ration effects on fat-corrected milk yields followed that of Trial 1 to the extent that the rations containing the higher amounts of energy (B and C) gave the higher yields. Ration C containing the lowest proportionate protein level with high energy gave the highest milk yields and weight gains. The yields from ration C exceeded those of ration D which contained over 20 per cent more protein with a small difference in the energy levels. The lower milk yields obtained with the high normal protein levels of rations D and A followed the same order as in Trial 1.

The relative and interaction effects of protein and energy on fat-corrected milk yields are shown in Table 6.

The trend here was similar to Trial 1 in that the ration level of energy had a highly significant influence on production. The reason why the effects of the level of protein in the rations were not significant could be because of the narrower range of protein levels as compared to those in the rations of Trial 1. Again the interaction of protein and energy was not significant.

The mean effects of rations on yields for Trial 2 are shown in Table 7.

Efficiency of Rations

As in Trial 1 an estimate was made of the protein in excess of maintenance requirements available for milk production. These values are as follows:

Rations	A	B	C	D
	lb.	lb.	lb.	lb.
Protein in excess of maintenance requirements	26.8	32.5	22.7	34.1
Protein in milk	14.8	15.7	16.5	15.7
Difference	12.0	16.8	6.2	18.4

TABLE 8.—INFLUENCE OF RATIONS ON MILK COMPOSITION

Rations / Treatment	A Normal protein-normal energy	B High protein-high energy	C Normal protein-high energy	D High protein-normal energy
	%	%	%	%
Trial 1				
Butterfat	3.86	3.82	3.83	3.86
Solids-not-fat	8.74	8.86	8.75	8.71
[1]Protein**	2.87	3.08	2.92	2.94
Trial 2				
Butterfat	3.54	3.49	3.50	3.64
Solids-not-fat	8.43	8.46	8.60	8.40
[1]Protein	2.87	2.82	2.86	2.91

[1]Protein = Nitrogen x 6.38
**Differences significant at P = 0.01 L.S.D. = 0.078

The trend in efficiency of the rations in this trial followed that of Trial 1 to the extent that the normal protein-high energy (ration C), indicated the most efficient use of protein and Ration D containing high protein was the least efficient. Ration C showed the least difference in the amount of protein available for milk over that contained in the milk produced and effected some gain in weight (Table 5). Ration D showed the largest excess of protein over that required for the milk and resulted in a slight loss in weight. Rations A and B were intermediate between rations C and D in this trial, without large discernible differences in their efficiency respecting protein utilization. This was probably because the differences obtained in the ratios of protein to energy fed in these two rations were not as large as for the respective rations in Trial 1.

Milk Composition (Combined Results)

The data for milk components –butterfat, solids-not-fat, and protein (N x 6.38)—from each trial were analysed by the analysis of variance method. The average percentages of milk components in order of rations for each trial are shown in Table 8.

Butterfat and solids-not-fat percentages were not significantly influenced by the rations fed during either trial. The effect of rations on the percentage of protein, determined from the nitrogen content of the milk samples, was highly significant for Trial 1. Rations B and D containing high protein levels showed a significantly higher percentage of protein or nitrogen than ration A containing normal protein and low energy. This trend is in agreement with the results of Perkins (12) who showed that feeding a high level of protein in the meal ration (Nutritive ratio 1:2) resulted in increased nitrogen in the milk. They found that the increase was due to increased residual or non-protein nitrogen. The true protein was not affected. It is not clear why the protein content of the milk from ration C was only slightly below that of ration D unless the high ratio of energy to protein in the ration may affect nitrogen secretion in the milk since the cows did lose weight while on this ration.

The differences in the percentage of protein or nitrogen in the milk from the respective rations in Trial 2 were not significant. This may be due to insufficiently wide ratios of protein to energy in the rations fed in this trial. There is an indication that the relatively high protein of ration D (Table 5) may have slightly influenced the percentage of protein in the milk.

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FLAVOUR STUDIES ON PORK FROM HOGS FED FISH SILAGE[1]

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ABSTRACT

Meat from hogs fed fish silage as a protein supplement was compared with that from hogs fed soybean oilmeal using the scores from a taste panel and from a thiobarbituric acid test as indices of its acceptability to the consumer. The tests were conducted upon non-cured shoulder cuts that had been frozen for storage and thawed just before oven-cooking.

A moderate off-flavour was detected by the taste panel on the meat from hogs fed fish silage. This off-flavour was greater on the fat portion of the meat than on the lean. The intensity of the off-flavours was not affected by the removal of fish silage from the ration of hogs at 170 pounds body weight as compared to the continuous feeding of fish silage to market weight. The off-flavour was considered sufficient to make the meat undesirable to the consumer.

The thiobarbituric acid test failed to show any differences between the fat from hogs fed fish silage and the fat from hogs that received soybean oilmeal.

INTRODUCTION

Large quantities of fish offal are wasted each year in coastal areas not serviced by fish meal plants. The manufacture of fish silage by preserving the offal in sulphuric acid and neutralizing the excess acid with limestone is an attempt to prevent this waste. The nutritional value of fish silage has been tested at the Nappan Experimental Farm and found to give satisfactory gains and feed efficiency with hogs when fed as a protein supplement (1).

In spite of the nutritional value of the product, its use in hog rations could be restricted because of off-flavours which may result in the meat (2). A taste panel test on meat from hogs fed fish silage has been reported by Seaman (3) but she did not consider the results conclusive or reliable. The present investigation was undertaken to obtain more conclusive data on the consumer acceptability of meat from hogs fed fish silage.

EXPERIMENTAL PROCEDURE

Meat samples were obtained from three groups of hogs, eight per group, which were individually fed in a trial at Nappan. The first group, Treatment A, was fed fish silage as a protein supplement until the animals reached 170 pounds in weight, then switched to a finishing ration without any fish silage. The second group, Treatment B, was fed fish silage throughout the trial. The third group, Treatment C, was fed soybean oilmeal as a

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protein supplement until the animals were 170 pounds in weight, then switched to the same finishing ration as fed to the animals in Treatment A. The fish silage (26 per cent total solids) was fed as a wet mash in the ratio of 1 to 2 with the basal grains until the pigs reached 105 pounds in body weight and in the ratio of 1 to 4 with the basal grains thereafter. The animals were slaughtered at 200 pounds body weight. A cut of meat was taken from the shoulder section of each animal after slaughter and frozen without curing. These samples were shipped to Ottawa by air in a dry ice pack and kept frozen until cooked.

Two methods, taste panel and chemical, were used to examine samples of meat for off-flavours.

Eight persons, none of whom was especially trained in the appraisal of meats, served as panelists. The panel met eight times, and examined three samples, one from each treatment, at each sitting. A sample of pork, considered representative of standard pork flavour, was given to each panelist at the start of each sitting. The conditions under which the taste panel test was performed, including such factors as coding of samples, time after smoking and the removal of flavour from the mouth, were those recommended by Dawson and Harris (4).

Meat samples were oven-cooked in an uncovered pan at 325° F. Eight slices were taken from each cooked sample and each slice divided into three parts so as to contain both fat and lean. The slices were randomly assigned to panelists and the slice parts were randomly assigned to the three replications at each sitting. The meat was warm when tested.

The panelists were requested to score the fat and the lean from each slice part with a score ranging from 5 to 0. The scores of 5, 4, 3, 2, 1, and 0 represented *no off-flavour*, *very slight off-flavour*, *moderate off-flavour*, *decided off-flavour*, *very decided off-flavour* and *excessive off-flavour* respectively.

For statistical analysis, the scores were converted to percentages and transformed with an angular transformation to produce an approximately constant error variance (5). An analysis of variance was made of both the fat and the lean meat scores.

The thiobarbituric acid test, successfully employed by Askoe and Madsen (6) to detect oxidized flavours in bacon fat, was used to grade samples of raw fat from each cut of meat. This test is based upon the detection of epihydrin aldehyde, a compound formed by the oxidation of fats. The data were analysed by analysis of variance.

RESULTS AND DISCUSSION

The mean flavour scores for both the fat and lean meat tested in the taste panel test are shown in Table 1. The mean optical densities determined by the thiobarbituric acid test also are shown in Table 1. An analysis of variance of the data is presented in Table 2.

The results of the taste panel test shows that the fat from hogs fed fish silage (Treatments A and B) received a lower score than did the fat from hogs receiving soybean oilmeal (Treatment C). The removal of fish

TABLE 1.—EFFECT OF FEEDING FISH SILAGE ON PORK FLAVOUR AND ON THE OXIDATION OF PORK FAT

Treatment	Mean flavour scores from taste panel test		Mean optical densities from thiobarbituric acid test
	Fat	Lean	Fat
A—Fish silage fed to 170 lb. body weight	3.2	3.7	.59
B—Fish silage fed to 200 lb. body weight	3.1	3.7	.62
C—No fish silage fed	4.0	4.3	.56

TABLE 2.—ANALYSIS OF VARIANCE OF TASTE PANEL SCORES AND OF OPTICAL DENSITIES FROM THIOBARBITURIC ACID TEST

Source of variation	Taste panel			Thiobarbituric acid test	
	Degrees of freedom	Mean square for fat	Mean square for lean	Degrees of freedom	Mean square for fat
Treatments	2			2	.0124[1]
A, B vs. C	1	26837**[1]	12661**[1]		
A vs. B	1	127[1]	5[1]		
Panelists	7	8805**[1]	9101**[1]		
Male vs. Female	1	49621**[1]	28826**[1]		
Sittings	7	1141[2]	601[2]	7	.1857**[2]
Replications	2	172[3]	42[3]	1	.0007[3]
Treatments × Panelists	14	1360**	310		
Treatments × Sittings	14	766*	450*	14	.0311**
Treatments × Replications	4	169	223	2	.0024
Panelists × Sittings	49	586*	293		
Panelists × Replications	14	298	101		
Sittings × Replications	14	271	120	7	.0053
Error	448	402	254	14	.0040

*Significant ($P < .05$)
**Highly significant ($P < .01$)
[1]Treatments × panelists mean square used in F test
[2]Treatments × sittings mean square used in F test
[3]Treatments × replications mean square used in F test

silage from the ration at 170 pounds in weight did not affect the flavour scores as compared to the continuous feeding of fish silage throughout the trial. Although the scores indicated only a moderate off-flavour due to the feeding of fish silage, this was considered sufficient to make the meat undesirable to the consumer.

The panelists differed widely as to their discrimination against the off-flavoured fat. The male members of the panel reported higher scores for

both the fat and the lean meat than reported by the female members of the panel. Variation was noted in what was actually termed off-flavour, some of the panelists detecting a fishy flavour while others could not. All of the panelists, however, reported that they were able to detect an off-flavour, whether it was fishy or not.

The differences between sittings and between slice-replication were not significant upon statistical test. The sitting mean squares are confounded with the animal replications from the feeding trial in this investigation.

The high interaction mean squares for treatment × panelists, treatment × sittings, and panelists × sittings, tend to indicate that the panelists lacked consistency in scoring the fat within the various treatments and at the different sittings.

The mean scores for lean meat from Treatments A, B and C were 3.7, 3.7 and 4.3 respectively. The analysis of these data reflect the same picture as that observed with the fat scores. The scores for lean meat from hogs fed fish silage were lower than those for the meat from hogs fed soybean oilmeal. The scores for the lean meat tended to be somewhat less variable than those for the fat, the coefficients of variation being 23.4 and 33.3 per cent respectively. The extent to which these lean scores are dependent upon fat contamination during the cooking process is difficult to ascertain. The higher scores for the lean meat do, however, show that the discrimination against the off-flavours in the lean meat was somewhat less than that shown against the fat. This finding is consistent with the results of Seaman (3).

Differences between the mean optical densities for Treatments A, B and C in the thiobarbituric acid test were not statistically significant. The level of optical density differed somewhat between the tests from one sitting to another, but these differences do not affect the validity of the tests between treatments. These results indicate the absence of off-flavours caused by oxidation of fat and suggest that off-flavours detected by the panel on the meat from hogs fed fish silage may have been due to some cause other than oxidation. This finding is contrary to those of Garton *et al.* (7) who noted that the differences between the fat from pigs fed whale-oil and that from normally fed hogs was the high degree of unsaturation of the fatty acids and the tendency toward rancidity.

The results of these tests indicate that the feeding of fish silage to hogs can cause a slight off-flavour in the meat which is sufficient to make it undesirable for marketing. Further investigation is needed to determine if discontinuance of fish silage feeding at a lighter weight or if curing and processing of the meat samples will affect the intensity of the off-flavours.

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